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Ministry of Higher Education & Scientific Research

University of Babylon

College of Science

Department of Biology

Investigation and Phylogenetic Study of Pin Worm *Enterobius vermicularis* and the Effect of Two Types of Plant Extracts on the Effectiveness of Worms *In Vitro*

A Thesis

Submitted by to the Council of College of Science , University of Babylon, in Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy in Biology

By

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Summary

The current study aimed to determine the epidemiology of *Enterobius vermicularis* pinworm among children from (1-15) years at the Babylon province and estimate the effects of extracts of Iraqi *Citrus limon* plant fruits and date vinegar in paralysis and death of *E. vermicularis In Vitro* , polymerase chain reaction (PCR) was used to detection the pinworm by using mitochondrial cytochrome oxidase subunit1 (Cox1) gene for local *E.vermicularis* isolates according to NCBI BLAST-related *E. vermicularis* isolates.

This study was applied on 972 children from the districts of Hilla center, province and civil hospitals, and health centers belonging to Babylon province from February 2021 to October 2021,the results showed that 664 (68.31%) children were infected with worm, there was no significant relationship between the gender of children and the infection, and the numbers of infected male children were 368 (55.42%), the numbers of infected female children were 296 (44.58%),there was a significant relationship ($p \leq 0.05$) between the habitat and the infection, the high percentage of infected children were from urban regions 495 (74.55%), while 169(25.45) infected children from rural regions, there was a significant relationship ($p \leq 0.05$) between the age groups and the infection, the higher percentage of infected children belonged to the age group of 8-10 years old 354(53.31%), while the lowest rate of infected children belonged to the age group 2-4 years.The results of extracts were appear that cold aqueous extract of *C. limon* plant fruits have a high influential especially by 300 mg/ml concentration where its average arithmetic for paralysis and die equal 29.00 and 57.33 minute , respectively, followed by hot aqueous extract of *C. limon* plant fruits in 300 mg/ml concentration where its led to paralysis and die at 67.33 and 104.00 minute ,respectively, followed by cold aqueous extract of date vinegar extract where its average arithmetic for paralysis and die in 300 mg/ml concentration equal 110.00 and 193.00 minute, respectively, albendazole drug successful through its high effect on parasite especially in 300 mg/ml concentration equal to 48.00 and 70.00 minute , respectively. Also a results of secondary compounds of *C.limon* plant fruits appears that a high effects of terpenoid compounds extract with 300 mg/ml

concentration where its led to paralysis and die of worms at 18.00 and 33.00 minute, respectively, while 200 and 100 mg/ml concentrations that led to paralysis and die at 27.00 and 46.67 minute ,respectively for 200 mg/ml concentrations and 38.00 and 65.00 minute ,respectively for 100 mg/ml concentrations, followed by phenolic compounds that led to paralysis and die of worms with 300 mg/ml concentration at 32.33 and 53.00 minute, respectively, as 200 mg/ml concentrations led to paralysis at 38.67 minute and to die at 64.00 minute, also 100 mg/ml concentrations led to paralysis at 70.00 minute and to die at 106.00 minute, followed by alkaloid compounds that led to paralysis and break of worms with 300 mg/ml concentration at 40.00 and 63.00 minute , respectively, also 200 mg/ml concentrations led to paralysis at 62.00 minute and to die at 95.00 minute, also 100 mg/ml concentrations led to paralysis at 75.00 minute and to die at 118.67 minute .

GC/MS technical results confirmed that Iraqi *C.limon* plant fruits contain limonene compound with a retention time of 17.651 minutes and a peak area of 0.71%, also, a consequence of limonene compound appears that it possesses a highly efficient in 400 µ /ml concentration where its average arithmetic for paralysis was 10 minute and for die was 30 minute, respectively, as well as albendazole drug, has a great effect despite a little number of concentrations especially in 400 µ /ml concentration where its led to paralysis at average arithmetic 145.00 minute and to killing at average arithmetic 243.00 minutes with significant differences. Molecular study results show that stool samples were positive for infection with worm, DNA for the cox1 gene was amplified to 407 bp, which was positive at agarose gel electrophoresis, the human *E. vermicularis* local isolates showed homology identity to the NCBI-BLAST *E. vermicularis* human isolates from Iran, the phylogenetic analysis was accomplished to construct the phylogenetic tree. The current study appears that a high endemic presence for infection with this worm in Babylon province also the plant extracts study appears that Limonene compound extracts of Iraqi *Citrus limon* plant fruits were the best from other extracts and the genetic study appears that local isolates were nearby from Iran *E. vermicularis* isolates .

وزارة التعليم العالي والبحث العلمي

جامعة بابل

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قسم علوم الحياة

دراسة استقصائية وجزيئية للدودة الدبوسية *Enterobius vermicularis* وتأثير نوعين من المستخلصات النباتية على فعالية الديدان خارج الجسم الحي

اطروحة

مقدمة الى مجلس كلية العلوم , جامعة بابل

وهي جزءا" من متطلبات نيل درجة الدكتوراه فلسفة

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1444 هـ **2022م**

﴿الْحَمْدُ لِلَّهِ الَّذِي خَلَقَ السَّمَاوَاتِ وَالْأَرْضَ وَجَعَلَ الظُّلُمَاتِ وَالنُّورَ ثُمَّ الَّذِينَ كَفَرُوا بِرَبِّهِمْ يَعْدِلُونَ﴾

صدق الله العلي العظيم

سورة الانعام / الآية (1)

Dedication

To the precious soul that left us and saddened us with their departure
To the *one who left a gap in my life that only he fills*
My father

My lovely mother, who motivated me….

My faithful husband, who supported and encouraged me....

My sister and my brothers for their

help….My children, who gave me the

hope…..

Alaa

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Alaa

Committee certification

We the examining committee, certify that we have read this thesis in titled "**Investigation and Phylogenetic Study of Pin Worm *Enterobius vermicularis* and the Effect of Two Types of Plant Extracts on the Effectiveness of Worms *In Vitro***" and have examined the student (**Alaa Hamady Obaied Hassan**) in its contents and that according to our opinion; it is accepted for the degree of Doctorate Philosophy of Science in Biology / Zoology.

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الخلاصة:

هدفت الدراسة الحالية الى تحديد وبائية الديدان الدبوسية *Enterobius vermicularis* ما بين الأطفال من (1-15) سنة في محافظة بابل , وتقييم كفاءة مستخلصات ثمار نبات الليمون العراقي*Citrus limon* وخل التمر على شل وموت هذه الديدان خارج الجسم الحي, تفاعل سلسلة البوليميراز(PCR) reaction Polymerase chain استعمل للكشف عن الدودة الدبوسية باستعمال جين الوحدة الاولى لانزيم السايتوكروم اوكسيديز المايتوكنديري للعزلات المحلية طبقا للعزلات العالمية المسجلة في بنك الجينات العالمي .

طبقت هذه الدراسة على 972 طفل من مناطق مركز الحلة والمستشفيات الحكومية والاهلية والمراكز الصحية التابعة الى محافظة بابل للمدة من شهر شباط الى شهر تشرين الاول2021 في مختبر الطفيليات المتقدم التابع لقسم علوم الحياة –كلية العلوم-جامعة بابل والنتائج الحالية للدراسة الوبائية بينت بأنه664 (68.31) طفل مصاب بهذه الديدان, لا توجد علاقة معنوية بين جنس الاطفال والاصابة , عدد الاطفال الذكور المصابين كان 368(55.42%) وعدد الاناث المصابات هو 296 (44.58).

هناك علاقة معنوية عند مستوى احتمال $p\leq 0.05$ بين المسكن والاصابة , النسبة العالية من الاطفال المصابين كانت من مناطق المدينة (74.55%)495 بينما (25.45%)169 طفل مصاب من المناطق الريفية .

هناك علاقة معنوية ما بين المجاميع العمرية والاصابة , النسبة العالية من الأطفال المصابين كانت تعود الى الفئة العمرية (8-10) سنة التي كانت (53.31%)354 بينما اقل نسبة من الاطفال المصابين هي تعود الى الفئة العمرية (2-4) سنة .

اظهرت نتائج المستخلصات بأنه المستخلص المائي البارد لثمار نبات الليمون يملك تأثيرا كبيرا خصوصا التركيز 300 ملغم/مل اذ كان الوسط الحسابي لشل وموت الديدان يساوي 29.00 و57.33 دقيقة على التوالي , يتبعه المستخلص المائي الحار لثمار نبات الليمون العراقي الذي يملك تأثيرا في شل وموت الديدان بتركيز 300 ملغم/مل اذ ادى الى شل وموت الديدان عند 67.33 و 104.00 دقيقة على التوالي , متبوعا بالمستخلص المائي البارد لخل التمر اذ كان الوسط الحسابي لشل وموت الديدان بتركيز 300 ملغم/مل يساوي 110.00 و193.00 دقيقة ,على التوالي ,يتبعه المستخلص المائي الحار لخل التمر اذ كان الوسط الحسابي لشل وموت الديدان بتركيز 300 ملغم/مل يساوي 139.00 و246.00 دقيقة على التوالي مع فروق معنوية واضحة , نجح عقار الالبندازول من خلال تأثيره العالي على الطفيلي اذ كان الوسط الحسابي لشل وموت الديدان بتركيز 300 ملغم/مل يساوي 48.00 و70.00 دقيقة على التوالي.

نتائج المركبات الثانوية لثمار نبات الليمون اظهرت التأثير الكبير لمستخلص المركبات التربينية لثمار نبات الليمون على شل وموت الديدان خصوصا بتركيز 300 ملغم /مل اذ ادت الى شل وموت الديدان عند 18.00 دقيقة و33.00 دقيقة على التوالي , بينما التركيز 200ملغم /مل و100 ملغم/مل ادى الى شل وموت الديدان عند 27.00 و46.67 دقيقة على التوالي للتركيز 200 ملغم/مل و38.00 و65.00 دقيقة على التوالي للتركيز 100 ملغرام/مل تتبعه المركبات الفينولية التي ادت الى شل وموت الديدان بتركيز 300 ملغم /مل عند 32.00 و 53.00 دقيقة على التوالي , التركيز 200ملغم /مل ادى الى شل عند 38.67 دقيقة والى الموت عند 64.00 دقيقة , التركيز 100ملغم /مل ادى الى الشل عند 70.00 دقيقة والى الموت عند 106.00 دقيقة تتبعه المركبات القلوانية التي ادت الى شل وموت الديدان بتركيز 300 ملغم/مل عند 40.00 و 63.00 دقيقة على التوالي والتركيز 200 ملغم/مل ادى الى الشل عند 62.00 دقيقة والى الموت عند 95.00 دقيقة والتركيز 100 ملغم/مل ادى الى الشل عند 75.00 دقيقة والى الموت عند 118.67 دقيقة .

اما نتائج تقنية ال GC/MS فأكدت بأنه ثمار نبات الليمون العراقي يحتوي على مركب الليمونين بوقت احتجاز 17.651 دقيقة ومساحة القمة 0.71% , اظهرت نتائج مركب الليمونين بأنه يملك كفاءة عالية عند تركيز 400 مايكرو/مل اذ كان الوسط الحسابي للشل هو 10 دقيقة وللموت هو 30 دقيقة على التوالي , عقار الالبندازول يملك تأثيراً كبيراً ً على الرغم من الكمية القليلة من التركيز على شل وقتل الديدان خصوصا التركيز 400 مايكرو/مل اذ ادى الى الشل عند وسط حسابي 145.00 دقيقة والى القتل عند وسط حسابي 243.00 د\قيقة مع اختلافات معنوية .

نتائج الدراسة الجزيئية اظهرت بأنه عينات البراز كانت موجبة للإصابة بديدان الدبوسية , دنا DNA جين Cox1 قد ضخم الى 407Pb زوج قاعدي الذي كان موجبا عند الترحيل الكهربائي بجل الاكاروز ,

العزلات المحلية للدودة الدبوسية للانسان اظهرت وجود تماثل مع العزلات البشرية للدودة الدبوسية الايرانية المسجلة في بنك الجينات العالمي, تحليل التطور الوراثي اجري لتكوين الشجرة الوراثية .تسلل العزلات المحلية اظهر بأنه يملك علاقة وراثية بالدودة المذكورة لبنك الجينات العالمي وباختلافات وراثية (0.0020-0.0080). الدراسة الحالية اظهرت وجود توطن عالي للإصابة بهذه الديدان في محافظة بابل وكذلك دراسة المستخلصات النباتية اظهرت ان مستخلص مركب الليمونين لثمار نبات الليمون العراقي هو افضل من باقي المستخلصات والدراسة الوراثية اظهرت انه العزلات المحلية قريبة من العزلات الايرانية لهذه الديدان.

Conclusions:

1-There was a high prevalence of *E. vermicularis* in Babylon province and a prevalence is not associated with a specific gender or while the majority of *E. vermicularis* infection was increased in urban regions. The 8-10 age group was the highest infected among the infected children age groups.

2-The cold water extract of *C. Limon* plant fruits has a high effect on paralysis and death of *E. vermicularis* worm more than its boiling water extract, then cold water extract and followed boiling water extract of veingar, respectively, *In Vitro.*

3-The terpenoid compounds of *C. limon* plant fruits with a high concentration has a high efficiency on paralysis and death of *E. vermicularis* worm, then phenolic compound followed by alkaloid compounds.

4-In this work, its demonstrated that GC-MS is an extremely accurate and technically advanced method for the isolation of components of secondary compounds, particularly the limonene compound, which is the most prevalent component of Iraqi *C. limon* plant fruit.

5-PCR is valuable for isolating and amplifying *E. vermicularis cox 1* DNA genes. The local isolates from pinworm-infected children showed 99% identity homology to the global NCBI-BLAST *E. vermicularis* human isolates from Iran.

6- The presnt study demonstrates that the limonene ompound had a highly efficient action against *E. vermicularis* adult helminths when tested in vitro.

Recommendations:

1- Conducting an educational health program on ways to spread this worm and how to treat them in the right ways through personal and public hygiene.

2- Conducting advanced studies about using terpenoid compound treatment of *E. vermicularis* worm.

3- Analyzing terpenoid compounds to their original combination and know which compounds are more effective against parasites.

4- Conducting advanced studies at the cellular toxicity level of this extract by experimenting with animals.

Supervisor's Certification

I certify that this thesis **(Investigation and Phylogenetic Study of Pin Worm *Enterobius vermicularis* and the Effect of Two Types of Plant Extracts on the Effectiveness of Worms *In Vitro*)** was prepared under my supervision at the Department of Biology, College of Science ,University of Babylon , in a partial fulfillment of the requirements for the degree of Doctorate of Philosophy of Science in Biology, and this work has never been published anywhere.

Professor
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/ / 2022

Recommendation of the Head of Department of Biology

In view of the available recommendations, I forward this thesis for debate by the examining committee.

Signature
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Head of the Department of Biology
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Date: / / 2022

1.Introduction:

Enterobius vermicularis, also known as Oxyuris, is a human intestinal parasite that causes disease and is a member of the nematode phylum Nematoda. Other names for this parasite include "threadworm," "seatworm," and "pinworm" (Wendt *et al*., 2019; Taylor *et al*., 2018).It is estimated that over 200 million people are infected with the pinworm, making it the most prevalent form of human helminthiasis. At rural areas, pinworm is more prevalent in children but has a low incidence at urban areas (Taylor *et al*., 2018; Meletis *et al*., 2019), pinworm is a health problem spread over the world(Dezsenyi *et al*., 2018).

Humans are a natural host of *E. vermicularis*, which is common in children and is related to high population density, socio-economic factors, and the habit of finger sucking (Presterl *et al*., 2019; Tomanakan *et al*., 2018). Eggs cannot survive in the environment for a long time, so the infections are limited in individuals who directly have contact with the diseases, such as school staff and nurseries, the primary ways of the condition are done by autoinfection by larvae or eggs, which accumulate in the anus reaching from fomites, door handles, bed sheets, contaminated hands, pyjamas, and eggs, and dust inhalation (Rudko *et al*., 2017; Taylor *et al*., 2018), *E. vermicularis* spread by fecal-oral route when a patient ingests eggs that are initially present in the perianal area of infested patients (Meletis *et al*., 2019).

Re-infection occurs easily in enterobiasis, and the prevalence of this infection is mainly dependent on public health and personal hygiene (Moosazadeh *et al*., 2017), retroinfection of the larvae through the anus and then to the colon is common (Chen *et al*., 2018).

E. vermicularis infection presents symptoms but can sometimes included the itching and inflammation of the vaginal and anal areas. The clinical signs include intestinal irritation, difficulty sleeping. However, some other *E. vermicularis* cases do not present any symptoms at all (Kubiak *et al*., 2017; Zouari *et al.,* 2018). Heavy infections may result in lack of sleep, decrease in body weight, increased hypersensitivity, clenching and grinding of the teeth, stomach discomfort, and vomiting. They can also lead to intestinal infection, bacterial infections, appendicitis, and abdominal pain (Taylor *et al*., 2018; Nias *et al*., 2019). After being consumed, the

eggs are discharged into the small intestine to form larvae and then develop into the adult stage in the colon and caecum. The pregnant female worms then travel to the perineum area at night to put the eggs (Tsai *et al*., 2017).

Diagnosis of *E. vermicularis* infection is performed by microscopic examination of the characteristic worm eggs, sample collection by swabbing from the anal area with cellulose tape before the defecation and before genital area washing in the morning (Dezsenyii *et al.,* 2018; AL-Jaf *et al*., 2018).The scotch tape method is very efficient for detecting *E. vermicularis* eggs the worms get out with stool (Niaz *et al*., 2019).

Molecular Diagnosis only in connection to the mitochondrial genetic materials of *E. vermicularis* is it possible to get comprehensive information about the DNA sequence (Tomanakan *et al*., 2018).The mitochondrial cox1 gene in pinworms is isolated from chimpanzees and humans, which revealed the presence of three distinct genetic types, which have been labeled as types A, B, and C. These genetic types were determined through the comparison of the sequences of the mitochondrial cox1 gene (Kubiak *et al*., 2017; Rollins, 2018). Only type A has been found in humans and chimps, whereas types B and C have only been found in chimps (Kubiak *et al*., 2017).

E. vermicularis DNA extracted from eggs is successfully amplified and sequenced to recognize this global parasite's genetic diversity, phylogeography and host specificity (Tomanakan *et al*., 2018), Human *E. vermicularis* is used in population genetics studies in modern populations, especially with the isolation of the mitochondrial *cox* 1gene. Prehistoric coprolites have been confirmed harbor this pinworm (Rollins, 2018). Mebendazole, albendazole, pyrantel embonate, and pyrvinium embonate are antihelminthic medicines that have been licensed, these antihelminthic medications have success rates of up to (90) %, they are ovicidal and adulticidal, and are thus regarded as a highly effective therapy. Mebendazole is the medication of choice for treating pinworm infections. Prolonged treatment for up to (112) days is advised for recurrent infections (Dezsenyii *et al*., 2018; Wendt *et al*., 2019),effective treatment of pinworm infection involves treating all close contacts (Taylor *et al*., 2018).

Numerous adverse reactions to the medicine, such as headaches, nausea, dizziness, a taste of metal, and treatment failures are recorded (El-Kutry and Sopeah 2020).

The medicinal plants generate a broad range of bioactive substances. It is a rich source of various medications; many plants have been used for treating illnesses in everyday life for many years in all parts of the world; these treatments have been implemented all over the globe (Phukan *et al*., 2017) , It has been said that medicinal plants are a part of nature's pharmacy. Since the beginning of human civilization, people have relied on plants for their care and various other needs. According to a survey conducted by the WHO, (70-80)% of the population in the world used plant medicines (Yamuna *et al*., 2017) . The secondary metabolites are an essential source of new drugs due to the wide variety of biomedical activities and chemical components they contain. Medicinal plants produce metabolites such as phenolics, lectins, polyphenols, essential oils, alkaloids, and terpenoids (Bergquist *et al*., 2017; Othman *et al*., 2019).

1.2.Aim of the study:

The study aims to conduct an epidemiological survey of pinworms in the province of Babylon and to know the effect of some plant extracts on these worms outside the living body in addition to genetic diagnosis by PCR and identify the genetic variations of these worms.

The study axis was:

1- Collecting samples from children under the age of fifteen years and diagnosing worm by PCR technology .
2- Extracting the active substances from date vinegar and *Citrus lemon* plant fruit by using one of the available methods and testing their biological efficacy against the parasite outside the living body, if possible as compared to the Albendazole drug
3- Diagnosis of the active compounds from the extracts under study by GCMS technology.

2.Literature Review

2.1. Historical Background

The discovery of a nematode parasite egg in coprolite in Brazil dating back 240 million years gives information on the pinworms, this result is especially interesting since it is the earliest record of an oxyurid nematode ever identified (Hugot *et al*., 2014).

E. vermicularis was first described by Karl Linnaeus in 1758, who named it *Oxyuris vermicularis* . Pinworms include *E. greggori* and *E. vermicularis* which most common parasites in human in the world (Gillespie and Pearson, 2001; Reinhard *et al*., 2016) . Paleoparasitological studies show the presence of pinworm eggs in human coprolites from numerous archaeological sites in Egypt, Iran, Korea, United States of America, Chili, Peru, and Brazil (Al-Samarai, 2020), It was discovered in ancient Egyptian mummified human remains, leading parasitology researchers to believe that it is the world's earliest known parasite. In addition to DNA samples from ancient human coprolite remains from both North and South America, the researchers also looked at samples from Europe and Asia (Satoskar *et al*., 2009; Reinhard *et al*., 2016).

In a second archeological site in Tehran city, the samples show the presence of one *E. vermicularis* egg from a female skeleton unearthed, samples of the soil were collected from the sacrum and pelvic bones, and this was evaluated to be 7000 years old. These findings may represent the oldest pinworm infection in a human to occur on Asia (Paknazhad *et al*., 2016). The microscopic examination of a coprolite from the Mid-West region of Brazil dated from the pre-Columbian period; confirmed the finding of *E. vermicularis* in a paleoparasitological analysis detected *Enterobius vermicularis* eggs that belonged to a human (Lino *et al*., 2018).

The samples from North American archaeological sites revealed the presence of *E. vermicularis* eggs that were identified in prehistoric populations of America (Al-Samarai, 2020). These findings demonstrate that ancient parasitism has substantial

utility in recording the breadth of human actions that impact parasitic illnesses (Reinhard *et al.*, 2016).

The positive samples in the American southwest archaeological sites date back 10,000 years in Chile and Peru, dating from 2200 to 400 BC (Ferreira *et al.*,2014; Reinhard *et al.*,2016). The parasitological and archaeological discoveries at a grave studied by Paknazhad *et al*.(2016) have revealed the earliest conceivable occurrence of a pinworm illness in this region of the globe, it also proved that humans resided in Tehran as early as the fifth millennium BC. Paleoparasitologists are able to detect ancient parasites of people and animals by using organic remains that have been discovered at archaeological sites, biological remains unearthed from archaeological sites which are the primary source of parasites in ancient times (Paknazhad *et al*., 2016; Lino *et al*., 2018).

2.2.Classification of *Enterobius vermicularis*

E. vermicularis was Classified by Karl Linnaeus in 1758 cited by Al-Samarai (2020).

Kingdom: Animalia Subkingdom: Metazoa

Phylum: Nematoda

Class: Secernentea (‘Phamidia’)

Order: Oxyurida (MBRAF)

Superfamily: Oxyuroidea

Family: Oxyuridae

Genus: *Enterobius*

Species: *E. vermicularis*

2.3.Morphology, Life Cycle and Transmission of *Enterobius vermicularis*

The adult worm of *E. vermicularis* has a thick external protective layer from cuticle

that is whitish-beige in color (Sumanto *et al*., 2021) .They are round in shape , with crawling movement, the head section of *E. vermicularis* is rounded , with a muscular bulb and esophagus, which is a characteristic that is unique to this worm (Sumanto *et al*., 2021; Wang *et al*., 2016).

The pinworm's mouth is bounded by three wing-like circular extensions called alae (Paniker and Ghosh, 2013). Female of *E. vermicularis*, measuring 8 to 13mm long by 0.4 mm wide, show distension of the body due to a large number of eggs in the uteri (Wang *et al*.,2016). Pinworms get their name from the enlarged tip at the posterior end of females, which gives them the appearance of pins (Wendt *et al*., 2019).The vulva opens into the single vagina between the first and second thirds of the body, and the single vagina leads to the paired uteri, oviducts, and ovaries. The vulva opens between the first and second thirds of the body (Zeibig, 2013; Paniker and Ghosh, 2013).

The double-layered, elongate-oval eggs are between 50 and 60 by 25 micrometers in size, translucent, and asymmetrical in shape. They have a thick shell and are convex on one side and on the other; they become infected within a few hours, and the egg's viability lasts for between two and three weeks (Taher, 2017; Wendt *et al*,.2019). Adult males measure 2-5 mm (Fan *et al*., 2019). Males are distinguished by having a single spicule that ranges in size from around 100 to 141 microns and has posterior ends prominently curled ventrally (Wendt *et al*., 2019).

Infection can spread from the nose to the mouth through contaminated hands, water, or food. Larvae emerge from eggs in the duodenum and become infective within 4-6 hours, then travel to the caecum to change and develop into mature (Figure 2-1) (Zouari *et al.*,2018; Sharma *et al*., 2018; Wendt *et al*., 2019) that are attached to the mucosa by their heads, and then the worms copulate (Wendt *et al*., 2019).

The males die after copulation; the females have a long life of more than one hundred days, then move to the anus (lay more than 11,000 eggs).However, females die or come back to the colon, the average period between the ingestion and the oviposition is (4) weeks (Wendt *et al.*, 2019; Khan *et al*., 2020; Sumanto *et al*., 2021).

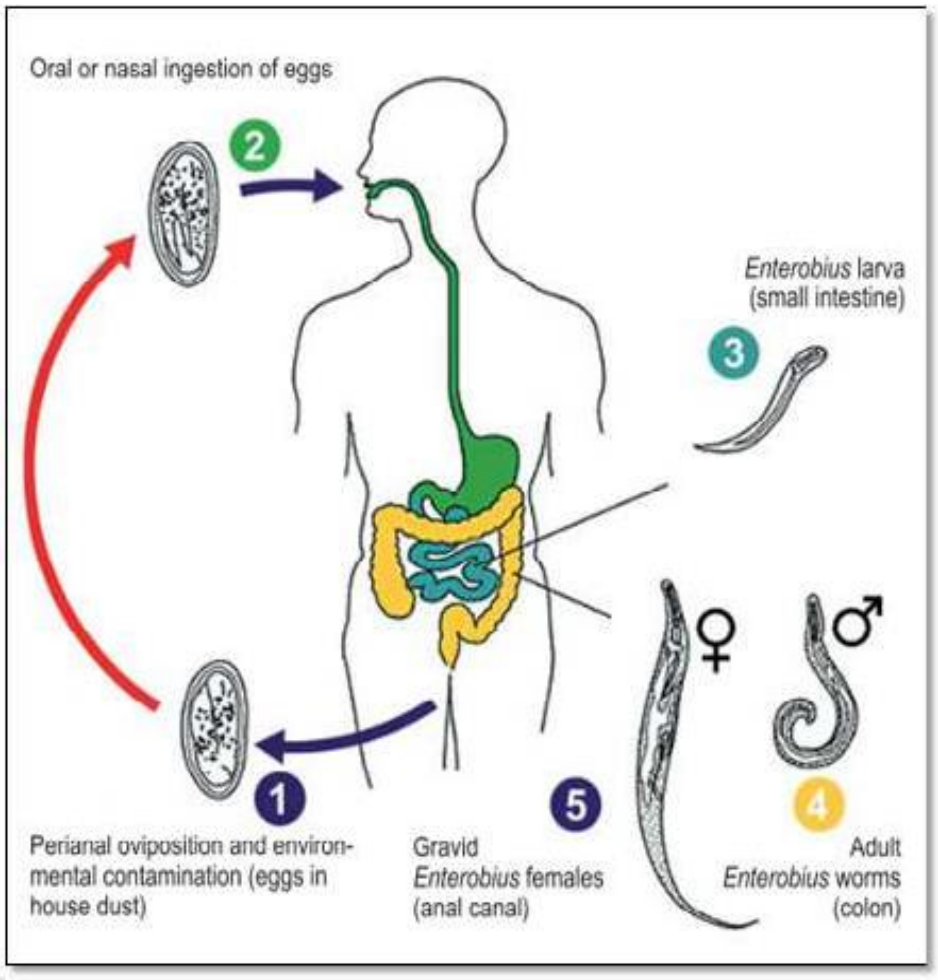


Figure (2-1): Life cycle of *E. vermicularis* (Wendt *et al*., 2019)

Pinworm transmition is done by ingestion of the eggs directly from the anus to the mouth, by the intense perianal itching, scratching of the affected area will transfer eggs to the finger and back to the original host, which is termed as autoinfection (Mehlhorn, 2016; khan *et al*., 2020), and it occurs due to nail biting, fingers sucking, inadequate hand-washing and poor hygiene (khan *et al*., 2020). Human acquire this parasite through direct contact with an infected person or ingestion of contaminated food and water and rarely by inhalation of airborne eggs (Dahal and Maharjan,2015;

Wang *et al.*, 2016) ,a retro infection also may occur when larvae migrate back after hatching into the large intestine (Santoshi & Krishnaiah, 2019; Wendt *et al.*, 2019).

The construction type of a bedroom of children and cleaning was not associated with a higher rate of infected individuals (Wendt *et al.*, 2019). Infection can also spread when people handle contaminated fomites such as tables, chairs, the ground, pajamas, bed linen and underwear (Chai *et al*., 2015; Fan *et al*., 2019), fruits and vegetables, particularly those eaten raw and without peeling, play a role in the transmission of *E. vermicularis* eggs, using of sewage on agricultural land and using this waste water for agricultural purposes well lead to cause fruit and leafed vegetable contamination with *E. vermicularis* ova (Rudko *et al*., 2017; Al-Mozan and Dakhil, 2019).

2.4. Epidemiology of *Enterobius vermicularis*

Pinworms are the most prevalent kind of human parasitic helminths; according to one estimate, there are around 200 million individuals infected with them all over the globe, with children between the ages of 5 and 10 accounting for more than 30 percent of cases (Fan *et al*., 2019). It tends to be more common in rural and poorer urban school children (Taylor *et al*., 2018).

There are numerous studies around the world to estimate the presence of *E. vermicularis* in society. In Pakistan, *E. vermicularis* prevalence was 2.8% of 2956 patients (Ahmed *et al*., 2015). A Prevalence of *E. vermicularis* among schoolchildren is investigated in Myanmar in three towns nearby Yangon city; the result revealed that of 761 children, 47.2% were infected with pinworms (Chai *et al*., 2015). Pinworm was detected in forty-four patients of the (438) students in Syria (Yazgan *et al* ., 2015). Enterobiasis among preschool children was determined in 2003 and 2013 in several cities in China; the prevalence in children was 12.75% and 5.13%, respectively (Wang *et al*., 2016). In north-eastern Poland, the prevalence of infection was 10.1% of a total of 296 healthy children (Kubiak *et al*., 2017). In Turkey, the rate of *E. vermicularis* was 38.6% of the total 357 children between the ages of 4-12 years (Babat *et al*., 2018).

Enterobiasis prevalence was determined in Iran with a percentage of (0.07%) in 292 patients in Sanandaj city in the center of Kurdistan province, northwest Iran (Bahrami *et al*., 2018). In Taiwan, *E. vermicularis* infection prevalence is extremely low in Taipei and of a total of 44.163 children(0.21%) were infected with pinworm infection among preschool children (Chen *et al*., 2018). In Thailand, the prevalence of *E. vermicularis* infections was investigated in children at Pathum Thani city, and a total of 397 children, 7.81% were infected (Taylor *et al*., 2018). The infection rate was reported among primary level government school children in Chhampi in Nepal. Out of the total of 107 children examined, 10.28% were positive for this nematode (Khadka and Maharjan, 2018). The prevalence of *E. vermicularis* in eastern Slovakia was 3.59% of the 390 children population (Dudlova *et al*., 2018).

In Bander Abbas, Hormozgan province, southern Iran, the prevalence of *E. vermicularis* was 1.6% in a total of 163 patients (Meskin *et al*., 2019). In Ethiopia, the prevalence of this nematode was 4.9% in the east and west Gojjam city (Asires *et al*., 2019). In Germany, *E. vermicularis* prevalence was detected between January 2007 and November 2017; this nematode was detected in17.4% of samples collected from 3991 patients (Friesen *et al*., 2019). In the Marshall Islands, the prevalence among preschool children was 22.4%, in 392 whose age ranged from 5-6 years (Fan *et al*., 2019), In Colombia, the prevalence of this infection was 6.15% in a total of 97 samples from children aged between 5-15 years (Hernández et *al*., 2019). The rate of *E. vermicularis* in children of the Mardan region in Pakistan, was recorded at about 34.05%, where that 370 children were examined and 126 were infected (Niaz *et al*., 2019). In İstanbul, Turkey, the Investigation of Intestinal Parasites in children studying in schools observed that *E. vermicularis* was 16.2% of 570 patients (Çakirlar *et al*., 2019).

In Nakhon Si Thammarat, Thailand, the overall prevalence of *E. vermicularis* infections among schoolchildren was (5.79%) in 23 of 397 (Laoraksawong *et al*., 2020). The rate of *E. vermicularis* among children of four regions in Pakistan was 5.75%, with 23 children in a total 400 (Khan *et al*., 2020). Etemadi and Hosniehoseini

(2021), Studied the Prevalence *E. vermicularis*, the rate of parasitic infection among children aged 3-10 years was 7.5% In the North-south and center of Zahedan in Iran.

As for the spread of these worms in the Arab world , there are some studies , Al-Nakkas *et al*. (2004) , studied the prevalence of *E. vermicularis* and found that *E.vermicularis* was (27.1%) among patients attending primary health care centers in Kuwait . In Al-Madina Al-Munawarah Governorate in Saudia Arabia, pinworm prevalence was estimated at 0.43%. Among expatriate workers (Taha *et al*., 2013). In the Gaza strip in Palestine, a histopathological survey was carried out among appendicitis patients that underwent an appendectomy in three hospitals; the result showed that 15.0% of patients are infected with *E. vermicularis* (Hamdona *et al*., 2013).

The prevalence of *E. vermicularis* also detected a 4.9% in Benha city in Egypt in a study that aimed to evaluate the degree of parasitic contamination of fresh vegetables consumed within Benha markets (Eraky *et al*., 2014). In Yemen, *E. vermicularis* prevalence among school children in all areas (urban and rural) of the Ibb city was 0.8% (Alsubaie *et al*., 2016), *E. vermicularis* was (0.28%) among expatriate workers in Sharjah, United Arab Emirates (Dafalla *et al*.,2017).The prevalence of enterobiasis infections in Kilowa Governorate in the Saudia Arabia Kingdom was estimated at 9% (Abd El-Latif, 2018).

A study in Hajjah governorate in Yemen revealed that this infection was 2.8% of a total 241 infected students in the primary school of this governorate (Alharbi *et al.*, 2019). The prevalence of *E. vermicularis* in expatriate workers in the United Arab Emirates in Al Ain City was estimated at 14.0 (Al‑Rifai *et al*., 2020).

Epidemiology of Enterobiasis in Palestine was reported by Hamarsheh (2021), a total of 29,061 (98.9%) in the West Bank region and 329 cases (1.1%) in Gaza Strip from 2008 to 2018.

In Iraq, there are a number of epidemiological studies, one of which was carried out by Hama and Rahemo (2014) in Erbil province; they showed that the incidence of

E. vermicularis was 29.8% among primary school children. In Al-Najaf province, evaluating the prevalence of *E. vermicularis* among children 4 to 7 years of age, the overall prevalence rate was high, 83.9 % (Hussein, 2015). Al-Saqur *et al*.(2016) studied the prevalence of *E. vermicularis* that was the most predominant helminths parasite (36,624) in all provinces of Iraq. A study of the incidence of helminthiasis in Iraq demonstrates that Enterobiasis is the most common helminthic disease 78,486 (Musa, 2017).

Another study in Sulaimania province in Kalar town among children revealed that the prevalence of infection was 24.9% of 1008 preschool and schoolchildren (Kadir and Amin, 2011). A high prevalence of *E. vermicularis* was recorded in Wasit Province, and 62% of cases gave positive results by direct microscopic examination, and 52% of cases gave positive results by Scotch tape (Rahi and Morad, 2017).

In a study aimed to evaluate the epidemiology of some parasitic helminths in Iraq in 2015, in a total of 56419 patients, 56206 patients were infected with *E. vermicularis*; the prevalence was 856 in Dahok, 71 in Erbil, 12 in Sulimaniyah, 596 in Ninewah, 460 in Kirkuk, 0 in Salahedin, 606 in Diala, 2148 in Baghdad rasafa, 7037 in Baghdad kerkh, 900 in Anbar, 510 in Wasit, 1119 in Babil, 415 in Karbala, 1230 in Najaf, 764 in Qadisyah, 960 in Muthana, 30504 in ThiQar, 40 in Missan, 7978patients in Basrah (Saheb *et al.,* 2017). The rate of infection percentage with *E. vermicularis* in Basrah city, south of Iraq was 3.21% among the patients that attended the Basrah General Hospital (Rhadi *et al*., 2018).

E. vermicularis infection rates were recorded in Al-Rusafa side of Baghdad city as 0.6% (Salman *et al*., 2019), in Al-Qadisiyah city, the prevalence of *E. vermicularis* was 34% (Alwaily *et al*., 2019), in Duhok province, north of Iraq, the majority of *E.vermicularis* was 18.01% among patients who consulted Hivi Pediatric Hospital (Hussein and Meerkhan, 2019). *E.vermicularis* was detected in 63 sample (13.26 %) in Mosul General hospitals and healthcare centers / Ninewah governorate, Iraq (Ahmed *et al*., 2020). The overall prevalence of *E. vermicularis* infection was 27.13%, among children in Erbil City (Al-Daoody and Al-Bazzaz, 2020),Al-Saqur *et al*. (2020)

mention that an *E. vermicularis* infection was (35.86) in Iraq during 2015 using an available database by the Ministry of Health from January 2015 to December 2015 of all provinces of Iraq. Al-Samarai (2020) estimated the spreading rate of *E. vermicularis* in Thi- Qar province among children, the results of his study showed that 53 (7.80%) of children showed infection of *E. vermicularis*. In Diwaniyah Governorate, Al-Ibrahimi (2019) examined (419) samples for children, the study results reveals that a number of *E.vermicularis* infected patients reached 183 out of 419 sample, with an infection rate of 43.67%. Al-Kinany (2020) recorded a high percentage of infection (73.5%) out of 200 samples in Wasit province also Al-Quraishi (2020) scored (41.11%) out of 135 samples in Wasit province.

In Babylon province there were some studies about infection of *E. vermicularis* such as Al-Morshidy(2007) he was investigate the prevalence of intestinal pathogenic parasites infections in Hilla city, results appears that *E. vermicularis* (2.4%), also in Babylon province Al-Taei, (2019) study recorded only 0.03% enterobiasis infection among seven years children.

2.5. Pathogenesis of *Enterobius vermicularis*

In adults, the infection may not produce any symptoms at all or may only manifest as minor symptoms (Kandi *et al*., 2019) . *E. vermicularis* does not have the buccal apparatus that helps penetration , but it still spreads hematogely through intestinal mucosal abrasions , also, it can reach many tissues such as the lungs and liver and cause threadworm granulomas (Dick and Hannay,2017) . Symptomatic and asymptomatic infections can occur in children and adults (Presterl *et al*., 2019).

However, less attention has been paid to *E. vermicularis* infection because the symptoms of Enterobiasis are seemingly not very severe (Li *et al*., 2015). Histopathology of the resected tissue revealed necrosis; in one area, *E. vermicularis* was present, the presence of structures resembling *E. vermicularis* adult worm and the eggs was observed during a pathological examination of a mass-like lesion (Kandi *et al*., 2019).

According to Saleem *et al*. (2017) findings, the patient was determined to have

severe acute and chronic salpingo-oophoritis with abscess development caused by *E. vermicularis* , All stages of *E. vermicularis* grow in the gastrointestinal tract; hence the host does not suffer from any systemic reaction until the worm burden becomes high or there is an ectopic infection, and as a result, many cases are asymptomatic . The worm triggers a mild, local inflammatory response, also when eosinophilic colitis has been described, circulating eosinophilia does not develop (Despommier *et al*., 2017).

The level of adenosine deaminase (ADA) is increased due to increased oxidative stress because of pinworm infection (Karaman *et al*., 2014).

In rare cases, *E. vermicularis* can lead to inflammation that will appear as ulceration in the colon, which may mimic tubercular ulcers, this ulceration occurs because of the worms attach themselves to the mucosa using their heads, and this is necessary for Pinworm to be invasive, two primary types of lesions are associated with tubular ulceration, ulcerative and ulcerohypertrophic (Mukherjee *et al*., 2015 ; Pehlivanoğlu *et al*., 2019). Pinworms mimic other serious diseases like colon carcinoma and Crons' disease (Johansson *et al*., 2013; Zouari and Mhiri, 2019).
It is believed that *E. vermicularis* cannot penetrate the intestinal mucosa unless there is some insult to the mucosal barrier (Mukherjee *et al*., 2015).

Parasitic infections should be considered in patients with acute appendicitis, and can cause inflammation in the appendix and imitate acute appendicitis clinically (Saravi *et al*., 2016; Tayfur and Balci, 2019). *E. vermicularis* can infect the female genital tract, and for anatomic reasons, it can migrate to fallopian tube, causing obstruction of one or both of them and leading to infertility (Obaid, 2018) . Central nervous system (CNS) infection can be caused by *E.vermicularis* ,this infection could stay for long due to the immune response, reinfection, malnutrition, bad hygiene or low socioeconomic status (Kandi *et al*., 2019), also can cause a significantly decrease in hemoglobin concentrations among infected children causing anemia with other low parameters of anemia, including packed cell volume (PCV) and red blood cells (RBCs) (Hama and Rahemo, 2014).

2.6.Diagnosis of *Enterobius vermicularis*

Eggs are laid by female worms on the skin around the anus. Some of these eggs may detach from the perianal area and get lodged on surfaces such as clothes, bedding, the ground, or tables and chairs (Fan *et al.*,2019; Kandi *et al*., 2019). Due to the difficulties in discovering pinworm eggs by stool tests, the rate of pinworm infection is likely underreported (Kubiak *et al*., 2017; Wendt *et al*., 2019).

2.6.1. Microscopic Examination

The presence of the typical worm eggs, which can only be seen under a microscope, is conclusive evidence of infection (Wendt *et al*., 2019). In contrast to other frequent gastrointestinal helminth infections, the intestinal infection is only detectable in 5% of cases with stool examination (Gunaratna *et al*., 2020). The diagnosis of pinworms differs from that of other intestinal nematodes since it is contingent on the demonstration of the eggs by the scotch tape test, which may be performed either late at night or very early in the morning (Hechenbleikner and Fascrs, 2015; Garcia *et al*., 2018).

Direct application and transfer onto a standard glass slide could submit the to microscopic examination for the diagnosis of the ova . In order to obtain the best possible results, specimens should be collected in the early morning hours before washing the perianal region (Kubiak *et al*.,2017; Wendt *et al*., 2019; Gunaratna *et al*., 2020) . Acellophane or scotch tape test technique is an excellent diagnostic approach for screening instead of stool tests since eggs can only be found in around 5% of fecal samples, the sensitivity of three successive tape collections is about 90% (Kubiak *et al*.,2017; Wendt *et al*., 2019).

Due to the poor invasiveness of pinworms, serological tests are not of any diagnostic importance. However, the eosinophilia showed elevated (IgE) levels in the serum (Wendt *et al*., 2019).

2.6.2.Molecular Diagnosis

Molecular–based approaches can also be used to diagnose this nematode,

traditional polymerase chain reaction (PCR), as well as real-time PCR, are powerful newer techniques which can improve sensitivity (Bharti *et al*., 2018). Also, molecular methods in the diagnosis of parasites are sensitive and highly accurate (Zhang *et al*., 2015) . So molecular tools might help to understand transmission routes and distinguish persistent from repeated infections (Zelck *et al*.,2011) . They can distinguish between strains of a single species, identify pathogens from non-pathogens, study parasitic genetics and virulence factors for pathogens that facilitate the identification and selection of appropriate treatment (Zhang *et al*., 2015). The new species-specific diagnostic PCR might identify more pinworm carriers than conventional tests (Zelck *et al*., 2011 ; Bharti *et al*., 2018).

2.7. Immunity Against *Enterobius vermicularis*

Exposure to pathogenic and commensal organisms highly influences the human immune system , the innate immunity that includes the intestinal mucosal surfaces , which contain a single layer of epithelial cells acts as a barrier between the host and the outside world (Barker, 2014). The survival of helminths in the host over long periods is the result of a process of adaptation or dynamic co-evolution between the host and the parasite (Motran *et al*., 2018) .Immune responses among host species vary with many other species , such as the environment and the life cycle phase (Al-Kabee *et al*., 2014) .The immune response caused by parasitic infection is complex and multiple (Hamid ,2019).

Eosinophil and IgE levels were higher in infected than in uninfected children, indicating immune response activation during infection (Patsantara *et al*., 2016; Kara, and Volkan, 2018) . The antibody level of IgE is higher in people with *E.vermicularis* parasite, and it are percentage is related to age and not sex (Hamid , 2019). Eosinophilia may be a characteristic of pinworm infections in pediatric patients, even in the lack of classic symptoms (Schroeder *et al*., 2019) . Damage to the host tissues caused by infection with helminth parasites produces the release of danger signals, which induce various cells, including innate immune cells such as macrophages, dendritic cells, eosinophils, basophils, and mast cells, these cells secrete soluble factors, which regulate the immune mechanisms that depend on the parasites, helminth

infections cause damage to the host tissues, which produces the release of danger signals (Motran *et al*., 2018).

2.8. Pharmacological Treatment

Since benzimidazole was used in such large quantities for more than three decades, there is a possibility that human helminths that are resistant to treatment may emerge as a result (Hong , 2018) , medical treatment of *E.vermicularis* infection includes albendazole mebendazole, pyrantel pamoate, and to a lesser extent flubendazole and ivermectin, the empirical use of anthelminthic drugs without medical supervision and low compliance to treatment protocols lead to resistance to therapy (Temsah *et al*., 2021).

2.8.1.Albendazole Drug

The efficacy and safety of albendazole (ADZ) made it used as an anthelminthic in humans for over 30 years; worldwide, ADZ is poorly orally absorbed (<5%), and high-fat meals enhance its uptake; ADZ is safe but not recommended for children <2 years or for women in the first trimester of pregnancy (Hong , 2018; Horton , 2020), in 1982, ADZ is a benzimidazole carbamic acid methyl ester which has anthelmintic and used in humans 1982 (Verrest and Dorlo, 2017; Horton , 2020). it is administered orally, either as tablets (200 mg or 400 mg) or as a suspension (2% or 4%), with the dose regimen dependent on the target parasite (McCarthy and Moore , 2020).

There are many of researchers studying activity of albendazole, Also in Calabar of Nigeria, Otu-Bassey *et al*. (2011) examined the connection between enterobiasis and enuresis in 632 children aged 5 to 14 years before and after treatment with albendazole, both enuretic and non-enuretic children were given a single dosage of 400 gm of albendazole over the course of the study, treatment of enuresis among patients with *E.vermicularis* and anal itching was 53.5% and 49.8%, while 34,3% and 24.9% among patients with no *Enterobius* and anal itching, respectively,

In Egypt Temsah *et al*. (2021) they have carried out a clinical trial that included 130 children (3-12) years old with resistant *E.vermicularis* infection, 36 out of 65 (55.4%) children in the albendazole treated group and 54 out of 65 (83.1%) in the

albendazole-flubendazole treated Group, they were cured with significantly higher efficacy for the albendazole-flubendazole Group.

2.9.Medicinal Plant Therapy

It's treating and preventing various diseases (Parthiban *et al*.,2015), and it's still have a positive future (Marrelli, 2021). Medicinal plants represent the oldest form of medication, used for thousands of years in traditional medicine in many countries around the world (Marrelli, 2021; Jamshidi-Kia *et al*., 2018),

it has a great ability to produce product metabolites such as phenolics, alkaloids, polyphenols, essential oils, and terpenoids (Othman *et al*., 2019); among the secondary metabolites studied, alkaloids and polyphenols have shown strong antimicrobial activity (Othman *et al*., 2019; Anand *et al*., 2019) . Also medicinal plants are gaining much interest recently because their use in medicine treating common diseases such as cold, fever, and other medicinal claims are now supported by scientific evidence (Azwanida, 2015), it is likely that humans also identified plants as antiparasitic drugs also infections by parasites are treated by secondary metabolites such as phenolics, terpenoids, and alkaloids (Wink, 2012; Klimek-Szczykutowicz *et al*., 2020; Gutiérrez-Grijalva *et al*., 2020).

Plant phenolics are natural secondary metabolites that arise biogenetically from either the shikimate route or another pathway, creating monomeric and polymeric phenols and polyphenols ,these phenols and polyphenols play important physiological functions in plants such as absorption of radiation (Reis-Giada, 2014), which are reported to have several effects (Elija *et al*., 2017),which are widely distributed secondary metabolites (Lin *et al*., 2015), they contain benzene rings, with one or more hydroxyl substituents and range from simple molecules to highly polymerized compounds (Velderrain-Rodríguez *et al*.,2014; Delgado *et al*.,2019).

Phenolic compounds could promote health benefits by reducing the risk of metabolic syndrome and they could contribute to the prevention of disease (Lin *et al*., 2015), these compounds are usually related to defense mechanism in the plant (Santos *et al*., 2020), it were widely distributed in plant foods (cereals, vegetables, fruits and

others), stressing among them the flavonoids, tannins, chalcones, coumarins, phenolic acids as benzoic acids and cinnamic acids, p-hydroxybenzoic, catechol gallic, vanillic, protocatechuic, lignins, and syringic acids (Giada, 2013; Lattanzio, 2013; Santos *et al*.,2020) .

Alkaloids are an organic substance which contain nitrogen atom one or more which usually situated in some ring system (Kurek , 2019), which mainly synthesized as secondary metabolites from many pathways, such as the shikimate, nicotinic acid, and lysine, and nicotinic acid pathway in plants, fungi, bacteria, and animals (Eguchi *et al*.,2019; Gutiérrez-Grijalva *et al*.,2020), their function in plants is not entirely understood but it is protect seed against predators (Gutiérrez-Grijalva *et al*.,2020), the nitrogen atoms of alkaloids cause alkalinity of these compounds (Kurek,2019), it is showed a strong toxic effects on animal and human in very small doses (Thawabteh *et al*.,2019; Kurek,2019), codeine, brucine, morphine and ephedrine represent as several alkaloids that have served as drugs and used in pharmacology (Othman *et al*., 2019 ; Gutiérrez-Grijalva *et al*.,2020), and has been efficiently utilized by human against bacteria , fungi and viruses (Thawabteh *et al*., 2019; Bhambhani *et al*., 2021).

Terpenoids are the hydrocarbons of plant origin, their molecules are consist of (2-5) carbon isoprene unit ways (Yadava *et al*., 2014; Prakash, 2018), terpenes are synthetized in the cytoplasm of plant cells through the mevalonic acid pathway (Rassem *et al*., 2016), most of the them are colorless, fragrant liquids which are lighter than water, a few of them are solids and all are soluble in organic solvent and usually insoluble in water (Yadava *et al*., 2014; Jaegera and Cuny, 2016), it were produced in diverse genera of plants, fungi, algae and sponges (Rassem *et al*.,2016), it can be divided into four groups of compounds that include true terpenes, steroids, saponins, and glycosides (Stephane and Jules, 2020), terpenoids, characterized by valuable pharmaceutical qualities and medical uses (Jaegera and Cuny, 2016).

Many of researchers studies anthelmintic activity of plant extracts on helminthes such as Sherwani *et al*. (2013) they were evaluated a anthelmintic potential of aqueous extract of *Cymbopogon citratus* plant (lemon grass) on *Pheretima posthuma*

earthworm in three concentrations 25, 50 and 100mg/ml ,the results indicated that100mg/ml lead to paralysis and death of worm in 62.43+ 0.11and 111.7+ 0.26 minutes, respectively, than other concentrations compared with Piperazine citrate as a standard in 100mg/ml concentrations which lead to paralysis and death of worm 8.84 + 0.03 and13.6+ 0.17 minutes, respectively, also Aziz *et al*. (2014), they were assessed anthelmintic activity of a methanolic extract of *Crinum latifolium* plant by five different concentrations (10, 20, 30, 40 and 50 mg/ml) on earthworms belonging to species *P. posthuma,* the lowest time for paralysis and death of worms at highest concentration (50mg/ml), were found 24±0.45 and 46.4±0.60 minutes, respectively,while albendazole, that used for resulting as the worm paralysis and worm death at 56.2 minutes and 77.4 minutes respectively, as well as Yang *et al*. (2014), he has investigated molluscicidal and larvicidal effects of linalool against *Schistosoma japonicium* schistosomula and cercariae in Balb/C mice, linalool exhibited the striking molluscicidal and larvicidal effects for cercaria and decreased the worm burden in infected animals.

In Al-Najaf province, Al- Madhi (2016) they were study an inhibition effect of hot and cold watery extract of clove *Dainthus caryophyllus* plant and albendazole on activity and life of cestodes worm *Raillietina* spp. that infected a pigeon *in vitro* ,the results showed that the 20 mg\ml concentration of hot watery extract has a clear effects on the worms after (3.3 ± 0.09) hours compared with control group (41 ± 1.41) hours and albendazole in same concentration that was (1.4 ± 0.11) hours.

Also Jatsa *et al*. (2018), they were study an effect of aerial parts *Sida pilosa* Plant on *Schistosoma mansoni* worms,aqueous extract was administered at 200 mg/kg to *S. mansoni* infected mice for 4 weeks, a group praziquantel infected mice were receiving a praziquantel at the dose of 100 mg/kg for 5 consecutive days followed by distilled water till the end of the experiment, aqueous extract induced a significant reduction of intestine enlargement hepatosplenomegaly and a number of granulomas was reduced by 52.82% in the liver and 52.79% in the intestine, whereas the volume of hepatic granulomas decreased by 48.76%.

According to Muluye *et al*.(2019),they evaluated a potential anti-malarial of an aqueous extract of the *Euphorbia abyssinica* plant on the *Plasmodium berghei* parasite, (25) Male *Swiss albino* mice were grouped into five groups of five mice in each group, group I was treated with distilled water (10 ml/kg), while groups II, III, and IV were treated with 200, 400, and 600 mg.

They studied the effect of the chloroform plant extract on a whole parts of the *Imperata cylindrica* plant against the tapeworm *Raillietina tetragona* and the roundworm *Ascaridia galli* in concentrations of 1.25, 2.5, 5, 10, and 20 mg/ml compare with the anthelmintic albendazole in vitro, this study was published as Lalthanpuii and Lalchhandama (2020), Both types of helminthes displayed abnormalities on their suckers, including clumping of the spines, tegumental folds, and erosion of microtriches, extensive damage was observed on the roundworm, including cuticular shrinkage, collapse of the lips, and formation of warty surface throughout the body.

2.9.1.Date Veingar

Vinegar having near about 5% acetic acid incorporated in water, traditionally vinegar implemented in food preservations applications (Hemke *et al*., 2019),the transformation of sugar present in fresh material to alcohol with inoculation of yeast, *Acetobacter* species are transformation of ethyl alcohol into acetic acid (Hemke *et al*., 2019; Gil *et al*., 2020),in a traditional method vinegar production takes elongated time interval for fermentation nearly about 30 days, acetic acid of vinegar act as food preservative (Chen *et al*., 2016; Hemke *et al*., 2019).The blood glucose control, lipid metabolism regulation, and weight loss capabilities from vinegars are mainly due to acetic acid (Chen *et al*., 2016).

Vinegar contains various nutrients and bioactive components include amino acids, sugars, vitamins, and minerals, acetic acid, gallic acid, catechin, ephicatechin, chlorogenic acid, caffeic acid, p-coumaric acid, and ferulic acid cause antioxidative, antidiabetic, antimicrobial, antitumor, antiobesity, antihypertensive, and cholesterol-lowering responses (Budak *et al*., 2014; Gil *et al*., 2020; Xia *et al*., 2020).

Acetic fermentation, is one of the most influential factors for the final physicochemical properties of fruit vinegars(Gil *et al*., 2020; Luzón-Quintana *et al*., 2021),vinegar is very popular as traditional ingredient for cooking, pickling, and preservation, it is made from sugar or starch by an alcoholic fermentation (Jamaludin *et al*., 2017 ;Gil *et al*., 2020), the contents of vinegars from sweet free amino acids are the main free amino acids followed by bitter amino acids (Wang *et al*., 2020).

The experimental study that performed in Iran by Sadjjadi *et al*.(2006) performed examination on giardiacidal activities by 1,000ml of lemon juice, vinifer and vinegar at 4C˚ and 24C˚ at 0, 0.5, 1, 2 and 3 hours on 250 cysts in each tube , they were showed that vinegar was more effective in inactivating *Giardia* cysts after 3 hours was 40.6% at 24C˚. , as in Turkey by Beyhan *et al*.(2016) they were investigates *Ascaris lumbricoides* eggs viability *in vitro*, it's were treated with 1, 3, 5, and 10% acetic acid concentrations in : 0, 10,15, 20, 30, 45, and 60 minutes, results appear that in 5%concentration at 30 minutes all eggs lost their viability.

2.9.2. *Citrus limon* Plant

The genus *Citrus* is composed of various species grown all over the tropical and subtropical regions (Kuo *et al*.,2017), it is a plant that belongs to Rutaceae (AL-Jabri and Hossain,2016), *C.* lemon tree is leathery and shiny with oil glands, it has joined in the stems, the blooms of this plant have five petals, and the aroma of its fruit is very sweet, the form of the fruit is a sphere (AL-Jabri and Hossain, 2016;Xinmiao *et al*.,2015).

C. lemon plant, cultivated worldwide due to its medicinal importance, is detected in most fruits and used as the medicinal herb to treat the diseases (Xinmiao *et al*.,2015), due to their active properties, citrus-derived secondary metabolites, such as flavonoids, limonoids, alkaloids, carotenoids, coumarins, and essential oils, are of vital importance to human health (Xinmiao *et al*.,2015; Elija *et al*.,2017),these active properties include antioxidative, anti-inflammatory, and anti-cancer effects, as well as cardiovascular protective effects and neuroprotective effects it is possible to draw the

conclusion that *C. limon* extracts have a significant amount of untapped potential as anthelmintic agents (Xinmiao *et al*.,2015; Huang *et al*.,2019 and Khatiwora, 2018).

There are many studies and experiments of anthelmintic activity of *C. limon* ,one of these studies take place by Khatiwora (2018) , he was evaluated the effect of Ethyl acetate, acetone, methanol extracts of *C. limon* leaves at 5, 10 and 20 mg/ml concentrations against Indian earthworm species *Eicinia foetida* parasite, where acetone extract exhibited highest activity than other the extracts,

As Upadhyaya (2018) who studied anthelmintic activity of *C. limon* peel against Indian Earthworm species *E. foetida* , Ethyl acetate, ethanol and methanol extracts at 5, 10 and 20 mg/ml concentrations were used, ethanol extract exhibited highest activity among the extracts, which has a time of paralysis 142 (min) while a time of death 64 (min), as Sravanthi *et al* . (2020) ,they were study anthelmintic activity of aqueous extract for *C. Limon* plant leaf on adult earthworm *E. Foetida*, in 3different concentrations (5mg/ml, 10 mg /ml and 20mg/ml), all the extracts exhibited better activity and produce paralysis as well as death of worms than standard albendazole, researchers Shija *et al*. (2020) investigated whether or whether the *C. limon* plant was efficient against malaria in mice that had been infected with *Plasmodium berghei* parasites the infected mice were separated into four groups, and each group received either 5% carboxymethyl cellulose / placebo, 0.2 ml *C. Limon* decoction extract alone, or a combination of artemether / lumefantrine (28 mg / kg) and 0.2 ml *C. limon* decoction extract and artemether / lumefantrine (28 mg/kg) as an oral dose ,all four groups were given the same total dose, in terms of hemato-immunological parameters indicate that the mice treated with the combination of artemether/lumefantrine and *C. Limon* extract had the highest mean counts.

2.10.Essential Oil

Essential oils (E.O.) are derived from plant material(De Groot and Schmidt, 2016a), can comprise between 100 and 250 components, and in some cases as many as 500 components , these components include limonene, B-caryophyllene, terpiens, and linalool (De Groot and Schmidt, 2016c).

E.O. are considered natural and unadulterated items, with peppermint, lavender, tea tree, and ylang-ylang being among the most popular (Sindle and Martin , 2020). During the manufacturing process, essential oils frequently go through a step called "post-treatment," which can involve the elimination of certain chemicals, the concentration of the oil, or a change in colour (De groot and Schmidt, 2016b). It has been discovered that the composition of essential oils is more complex than was previously believed (Sindle and Martin, 2020), the final composition of each oil may be different from one another depending on the harvest year, the country of production, and the manufacturing procedure (De Groot and Schmidt, 2016b). It is often discovered in medicines, many of which are suggested by medical professionals due to the analgesic, antipruritic, cough suppressant, and decongestant qualities that it has (Sindle and Martin, 2020).

There are many searches and experiments of anthelmintic activity of E.O such as Zenner *et al*.(2003), essential oils obtained from fresh leaves of *Cinnamomum aromaticum, C. limon* pericarps and *Allium sativum* bulbs was investigated *In Vitro* on *Tetratrichomonas gallinarum* and *Histomonas meleagridis*, a minimal lethal concentration (MLC) on *T. gallinarum* at one day was 0 .2 5 µl/m l for *C. aromaticum* oil, 0 .1 2 5 µl/m l for *C. limon* and *A. sativum* oils. On *H. meleagridis,* MLC was 0 .5 µl/m l for *C. aromaticum* oil and 1 µl/m l for *C. limon* and *A. sativum* oils at 2 4 and 4 8 hours.

Jaradat *et al*.(2016) carried out anthelmintic assay on adult earthworm *P. posthuma* by *Thymus bovei* plant E.O. at different concentrations (10, 40, 50, 75, and 100 mg/ml), and demonstrated that *T. bovei* E.O.s showed an anthelmintic effect, at the same concentration of 10 mg/ml, the time of paralysis was 19.61 and 24.25 min for the essential oil and piperazine respectively, while the time of death for essential oil and piperazine was 47.32 and 62.96 min. , respectively. Also Al-Hilfi *et al*. (2021) studied the effectiveness of lemon oils against *Balantidium coli* parasite; a crude hexane extract was examined onto a water agar medium that contained *B. coli*, a results showed high effectiveness of lemon oil within four days where FTIR spectrum of

lemon oil shows 1750 nm compared with a Metronidazole where FTIR spectrum of it appear 10250 nm while FTIR spectrum of *Mentha piperita* oil shows 2250 nm.

2.11. Gas Chromatography-Mass Spectrometry (GCMS) Technique

Gas chromatography equipped with mass spectrometry (GCMS) is a technique that separates complex mixtures into their individual components for identification and quantification (Jackie *et al*., 2020); it is used platform for analyzing volatile substances (Wu *et al*., 2014), quantitative composition information of the herb investigated could be provided by GCMS, which will be extremely useful for the further research for elucidating the relationship between chemical constituents in the herbal medicine and its pharmacology in further research (Yamuna *et al*., 2017), also, most of them can provide a rapid qualitative function based on the reliability of a chemical database, and the quantification may be made more accurate by combining the use of isotope standards and chosen ion mode (Wu *et al*., 2014; Jones, 2019).

MS detector gave high sensitivity and strong selectivity in samples analysis (Wu *et al*., 2014), extensive GCMS database, such as a library, paired with a retention index presented a credible approach to identifying substances in a complex combination (Wu *et al*., 2014; Jones, 2019).

Using GCMS for the analysis of herbal medicines offers at least two significant benefits: first, with the capillary column, GCMS has in general very good separation ability, which can produce a chemical fingerprint of high quality, and secondly, with the coupled mass spectral database (Yamuna *et al*., 2017).

2.12. Limonone Compound

Limonone are naturally secondary metabolites found in plant species of the Rutaceae and Meliaceae families whose antioxidant activity (Zandalinas *et al*., 2017; Ibáñez *et al*., 2020), limonene, mainly found as a major component in *Citrus* species which possess a valuable potential in synthetic pesticides , food preservatives also as antimicrobial, herbicidal and antioxidant agent (Ibáñez *et al*., 2020),which consist of 4-isopropenyl-1-methylcyclohexene (Soulimani *et al*., 2019;Ibáñez *et al*., 2020).

It has been found in more than 300 essential oils and principally in *Citrus* species (30–98%) (González-Más *et al*., 2019; Mahato *et al*., 2019), it occurs as two optical isomers, named d- and l-limonene (Ravichandran *et al*., 2018; Klimek-Szczykutowicz *et al*., 2020), the most common, d-limonene is obtained from the peel oil of these *Citrus* fruits in which its concentration can reach up to 97% by weight (Soulimani *et al*., 2019; Mahato *et al*., 2019), whereas, l-limonene is more present in other species such as *Mentha* species essential oils (Soulimani *et al*., 2019;Pang *et al*., 2019),both are additives in cosmetics, food, industrial solvents and pharmaceuticals because of their fragrant and harmlessness for humans (Vieira *et al*., 2018;Soulimani *et al*., 2019 and Ibáñez *et al*., 2020**),** *C. limon* peel extracts are found to do superior anthelmintic agent then the standard compound albendazole (Upadhyaya, 2018; Soulimani *et al*., 2019).

2.13. Molecular Characterization

2.13.1. Phylogenetic of Nematode

Molecular tools are recently been applied for the classification and study of the genetic diversity of parasitic helminths (Weerakoon and McManus, 2017), such as mitogenome is molecular technique for inferring nematodes phylogenies (Kern *et al*., 2020), one of the mitochondrial genes was cox1 gene revealed considerable genetic diversity in phylogenetic analysis (Tomanakan *et al*., 2018), it has various advantages when used as a phylogenetic marker, and it has produced good findings that were not well resolved when other markers were used (Kern *et al*., 2020).

Nematodes are among the most diverse and abundant metazoans on earth; however, research on them has been biased toward parasitic taxa and model organisms (Smythe *et al*.,2019), even though the use of mitogenomes is an effective tool for nematode phylogenetics and has been frequently employed to resolve ambiguity within this Group, the fact that mitochondrial and nuclear gene trees do not coincide calls for more research (Kern *et al*.,2020 ;Smythe *et al*.,2019), nematode Gene order may also be employed as a source of phylogenetic information in worms, accurate phylogenies can offer unambiguous information on the evolution and diversity of the Nematoda

(Kern *et al*.,2020),the phylogenetic analysis of DNA sequences revealed the existence of three distinct clusters, which were distinguished from one another based on the sorts of amino acid sequences they contained: A, B, and C. (Tomanakan *et al*.,2018).

2.13.2. *Enterobius vermicularis* Phylogenetic Studies

E. vermicularis of the order Oxyurida have a long evolutionary history with humans,

occurring only in animals that are not carnivorous (Hugot *et al*.,2014; Reinhard *et al*.,2016), many parasites have developed a number of cellular adaptations to them, including modification of genomes and organelles (Walochnik and Duchêne, 2016).

The phylogenetic tree based on *cox*1 gene variability showed possible close relationships between *Enterobius pongoi* and *Enterobius hugoti*, these species could have initially diverged in sympatry from a common ancestor (Foitová *et al*.,2014).

The phylogenetic analysis of mitochondrial co*x*1 and chromosomal 18 subunit ribosomal DNA(18S rDNA) and internal transcribed spacer 1 (ITS1) regions could support the independent status of several Nematoda species (Foitová *et al*.,2014; Rollins, 2018), but the genetic characterization of the internal transcribed spacer 2 (ITS2) region of nuclear ribosomal DNA (rDNA), the mitochondrial *cox*1 gene and ITS2 genes of *E. vermicularis* showed that the *E. gregorri* is synonymous to *E. vermicularis* (Rollins, 2018).

2.13.3. *Enterobius vermicularis* Mitochondrial Genome

Mitochondrial genome information such as gene arrangement, nucleotide, and amino acid sequences, used in the phylogenetic marker for investigating relationships at different taxonomic levels of diverse living organisms (Figure 2-2) (Hegedusova *et al*. 2014; Tanaka *et al*. 2014). Therefore, mitochondrial genes and whole mitochondrial genome sequences are widely used as molecular markers in studying population genetics (Kim *et al*., 2018).

The nematode mitogenome is typically a single, circular DNA molecule that ranges in size from 12 to 22 kb and contains a total of 36 genes (Rollins, 2018; Kern *et al*.,

2020), according to Rollins (2018), the gene organization of *E. vermicularis* is completely and unquestionably different from that of the vast majority of other nematodes that belong to the class Chomadorea, which are roundworms.

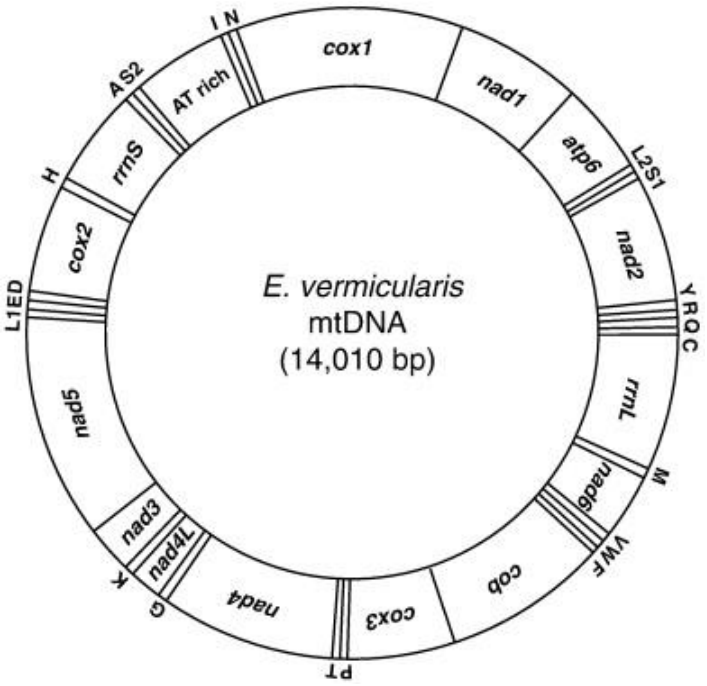


Figure (2-2) *E. vermicularis* mitochondrial genome (Kang *et al.*, 2009).

2.13.4. Cytochrome Oxidase 1 (*cox1*) Gene

The results of a phylogenetic study of the *cox1* gene indicated three clusters, each with a high bootstrap value, this indicates that the human pinworms have undergone significant divergence, which is likely connected to the evolution of humans (Nakano *et al*.,2006; Kubiak *et al*., 2017), it appears to be a considerable genetic diversity in phylogenetic analysis (Tomanakan *et al*., 2018), where the isolates were different in terms of the sequences within the *cox1* gene, also, mitochondrial genes sequencing analysis are a preferable method for direct genotyping of *E. vermicularis* from clinical samples (Hagh *et al*., 2014; Kubiak *et al*., 2017).

There are a number of studies about phylogenetic analysis of mitochondrial *cox1* gene of *E. vermicularis* in worldwide, a study of *cox1* genotypes was performed on a human population in Greece with 333 long nucleotide fragments and all samples clustered within the previously reported haplogroup B (Piperaki *et al*., 2011), as

another study on Danish and German patients also found a homogenous population of the B haplogroup using 333 nucleotide long fragments of the *cox1* gene (Ferrero *et al*., 2013).

Also study by Foitová *et al*. (2014) in Indonesia on phylogenetic tree based on *cox1* gene ,they were showed potential related between *Protenterobius hugoti* and *P. pongoi* ; these species have initially diverged in sympatry from a common ancestor, as well as in Iran Hagh *et al*. (2014), performed a study to investigate the existence and distribution of different *E. vermicularis* genotypes based on mitochondrial *cox1* gene , a results showed that *E. vermicularis* type B includes 2 subtypes including B1 and B2 a 379 nucleotide fragment revealed that the all samples belonged to haplogroup B and twenty and twenty five of them samples were subtype B1 and subtype B2, respectively, also Kubiak *et al*. (2017) done a research in Poland for molecular analysis of adult female *E. vermicularis cox1*gene, findings appears existence of a three different haplotypes of Pinworm, all sequences clustered within type B, together with human *E. vermicularis* isolates from Denmark, Germany, Greece, and Japan.

Another study conducted by Tomanakan *et al*. (2018), they were identification a genetic variation of *E. vermicularis* eggs in Thailand from schoolchildren using sequence analyses of the mitochondrial *cox1* gene, phylogenetic analysis of *cox1* sequences showed 66 haplotypes, Six haplotypes from Thailand which type in A (along with sequences from Japan and Korea) and five haplotypes into type B (with sequences from Japan, Iran, Czech Republic, Greece, Denmark, and Sudan), as Tavan *et al*.(2021), in Iran, they have collected 1 000 samples, mitochondrial *cox1* gene has a sequenced isolate belonged to *E.vermicularis* genotype B.

In Iraq, there are several studies for phylogenetic analysis of *E. vermicularis* depending on the mitochondrial cox1 gene and molecular diagnostic using PCR, as Al-Ibrahimi (2019) in Al-Diwaniyah province, she was accumulated (419) random stool samples for children aged (1-12 years), her results appear, bands of *cox1* gene of *E.vermicularis* parasite based on the 301pb which amplified by PCR methods ,as well as in Thi-Qar governorate, Al-Samarai (2020) study the molecular characterization of

E. vermicularis to determine the genetic variation, this studies was applied on 681 children their age less than ten years , PCR products of *cox*1 gene in *E. vermicularis* showed that 28 (52.83%) of samples were positive at 566 bp product size.

3. Materials and Methods

3.1. Materials and Equipment

3.1.1. Equipments and Apparatus

The equipments which used in this study presence in table 3-1 as follow:

Table (3.1) The equipments that used in this study and its manufacturer and state .

No	The equipment	Company and Country
1	Autoclave	Binder (USA)
2	Camera	Nikon/Japan
3	Centrifuge	Binder (USA)
4	Clevenger device	Quick fit (Germany)
5	Electric grinder	PA-B300C3 Royal (Japan)
6	Electrophoresis	Bioneer/ Korea
7	Eppendorf tubes	Biobasic/ Canada
8	Exispin vortex centrifuge	Bioneer (Korea**)**
9	Gas chromatography and mass spectrometry	California (United States)
10	High speed Cold Centrifuge	Eppendorf (Germany)
11	Incubator	Memmert (Germany)
12	Micropipettes (different volumes)	Eppendorf / Germany
13	Microscope	Smitech XSZ-N 107 (Malaysia)
14	Oven	Binder (USA)
15	PCR thermocycler	Bio-Rad in the United States
16	Reflex condenser	(Quick fit Germany type)
17	Refrigerate	Concord/ lebanon
18	Rotary evaporator apparatus	Oren scientific Ltd.(USA)
19	Sensitive Balance	Ohaus /USA
20	Soxhlets apparatus	Quick fit (Germany).
21	Spectrometer (Nanodrop)	Thermo Scientific (UK)
22	T100 Thermal cycler PCR	Bio-Rad/ USA
23	UV Transilluminator	Wised (Korea).
24	Vortex	CYAN (Belgium)
25	Water bath	Kottermann (Germany)

3.1.2. Chemicals

Chemicals which used in this research and its state and manufacturer company as in table (3.2)

Table (3.2)The chemicals which used and its manufactured company and the state .

No.	Chemical	Company and Origin
1	Absolute Ethanol	BDH (England)
2	Agarose	iNtRON (Korea)
3	Albendazole drug	Egyptian international pharmaceutical industries company(Egypt)
4	D-limonene	FedEx company (China)
5	DNA Marker ladder (100bp)	iNtRON (Korea)
6	Ethidium Bromide 10mg/ml	BioBasic (Canada)
7	Free nuclease water	BioLabs (UK)
8	TBE buffer10x	iNtRON (Korea)
9	Tween 80	FedEx company (China)

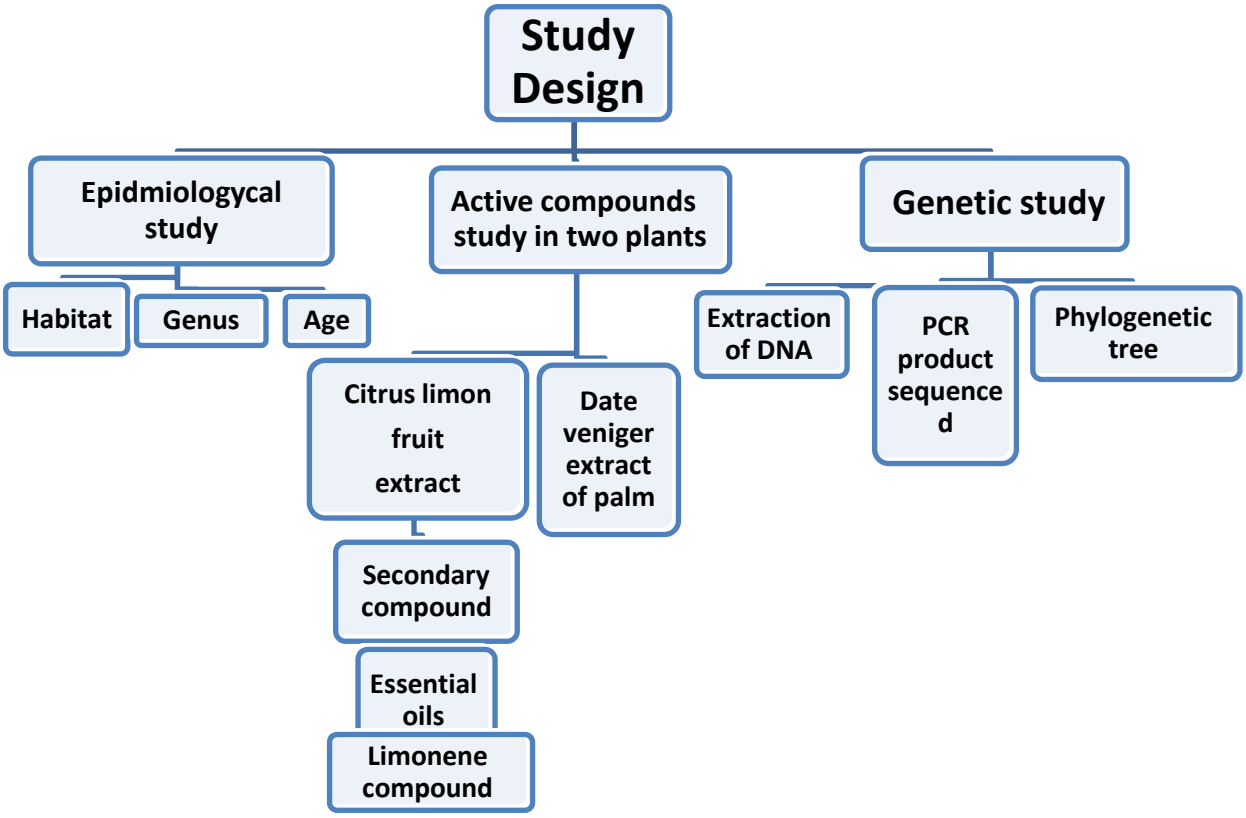


Figure (3.1)Experimental design of present study

3.2.Epidemiology Study

This study was applied on 972 children from the districts of Hilla center , Government and civil hospitals and health centers belonging to Babylon Province of Iraq for the period from February 2021 to October 2021.

3.2.1. Test Solution's

3.2.1.1. Lugol's Iodine Stain

Solutions were prepared from a following substances (10g. of Potassium iodide (KI), 5 g. Iodine and 100 ml. Distilled water**)** . Dissolve Potassium iodide in (25) ml. of the water, Heating after adding iodine with mixing. Then Dilute by the distal water to (100) ml. (Bryce and Poelma,1998 ; Al-Esawi , 2010).

3.2.1.2. Phosphate Buffered Saline (PBS)

Phosphate-buffered saline (PBS) is an isotonic solution has pH(7.2- 7.4), that was used in kept a live worm , it was prepared from a following substances **(**8 g. of NaCl,2.89 of $Na_2HPO_4.7H_2O$,0.2 g. of KCl , 0.2 g. of KH_2PO_4 and 1L. of distill water) after dissolve all substances in certain volume of water , ending a volume was completed to one liter after this sterile in autoclave at 121 c^o under 15 pound /Ang^2. for 15 minutes(Hudson and Hay,1984) .

3.2.1.3. Normal Saline Solution

Isotonic solution used during examine stool sample, It was prepare by dissolve 9 gram of Nacl with one liter of distal water where Nacl dissolve in a quantity of water then ending a volume was completed to one liter for concentration become 0.0009 gm/ml, after this sterile in autoclave at 121 c^o under 15 pound /Ang^2. for 15 minutes then kept in refrigerate at 4 c^o (Collee *et al*.,1996).

3.3: Collection of Stool Sample:

The assembly of the specimen is dependent on the presence of certain symptoms of the disease, such as the presence of anal itching during the night, abdomen pain, appetite loss, urinary tract infection, weight loss. Samples were collected in plastic containers clean court, with an emphasis on collecting exit samples in the morning. Additionally, the adhesive tape method was used to collect pinworm eggs and diagnose worms. The positive samples for molecular study were stored by freezing at -20 °C to preserve them for molecular analysis (Garcia *et al*., 2018; Al-Jaf *et al*, 2018).

3.4: Microscopic Assay

The stool samples were examined by microscope after collecting directly in 30 minutes and screening for presence of ova stages at parasitology Laboratory of biology department, Science College, University of Babylon as the following:

3.4.1: Lugol's Iodine Smear

The stain Lugol's Iodine is prepared to be applied with wet mount compensation and concentration procedures for the detection of intestinal helminthes ova .

1. Iodine stain diluted 1: 5 with distal water. (Every 3 weeks the working solution should be prepared) Fresh.
2. Physiological (0.85%) saline on a clean glass slide and a limited portion of stool (200 mg) mixed with a drop of sterile.
3. Put cover slip over the sample and examine the preparation of wet mount for the presence of ova.
4. Examine the slide under microscope for presence ova or adults parasitic structures .
5- Put iodine on the slide and limited portion of stool (200 mg) mixed with a drop of sterile (Al-Quraishi , 2006 ;Al-Jaf *et al*, 2018).

3.4.2: Scotch Tape Method.

Scotch tape was distributed on families that have infected children and a sample was collected from children in the early morning before the child take a bath or goes to the toilets by their mother. The tape is put on the perianal and pressing areas 3-4 time for each test, then the tape is placed on labeled glass slide, at the laboratories the collected labeled slides transferred and examined by the microscope, under 4,10 and 40 magnification power respectively (Lee *et al*., 2002).

3.5. Plant Extracts Effects Study

3.5.1. Plant Materials Collect

The of fruits of Iraqi *C. limon* plant and date veingar were collected from the local markets at February 2021. A sample of *C. limon* plant and date veingar was desiccants in shade position and then grinding by electric grinder to get on an soft powder which kept in plastic plate and then save in refrigerator.

3.5.2. Preparation of the Cold and Hot Aqueous Plant Extracts

Cold aqueous extract was attended by take 10 grams from the all parts of fruits powder of the *C. limon* plant and put it with 200 ml. of distilled water in the flask

400 ml. in size with shake a solution for 30 minute , then put in the test tube in the centrifuge for 10 minutes at 3000 p.m / minute , top was taken and left the sediment that collect and dried by putting it in the oven at 45 c^{o} to obtain on dried extract , keep it in the fridge until use. Date veingar powder was directly used for prepare cold and hot aqueous plant extracts. Hot aqueous extract for these plant was prepare by a same method but use boiling water (Al-Minsouri,1995) .

3.6. The Effects of Aqueous Plant Extracts on Adult *E. vermicularis* Worm *In Vitro*

Preparation of stock solutions of an cold and hot aqueous extracts of the *C. limon* plant fruits and date veingar which brings by melting (2) g. of dry extract in (5) ml. of distilled water. The stock solution become (400) mg. /ml. Stock solutions are used in several concentrations (100,200 and 300mg /ml). The control is prepared from PBS only . Four groups of worms , each group with five worms one of them representing a control group , each group have been treated with desired concentration of extracts and drug.

The worms were assembled in petridishes which contain one ml of PBS then put in an incubator , after one hour, different concentrations of cold and hot extracts for *C. limon* plant fruits and date veingar have been added to petridishes that contain worms each on its own by adding one ml. of each concentrations to a petridishes that contain parasite each on its own (Al- Madhi, 2016). The amount of time needed for full paralysis and death was measured and recorded for all three duplicates. In order to determine how long the paralysis would last, external cues were used. The amount of time it took for the worm to stop moving was measured as the paralysis time, and the amount of time it took for the worm to die, followed by a change in the body colour to become dark beige colour , was used to determine the lethal time (Al- Madhi, 2016; Khatiwora, 2018).

3.7. Extraction of Secondary Plant Component

3.7.1. Extraction of Crude Alkaloids

10 g. of dehydrated soft powder is extracted by putting it in the filter papers which is fixed on thimbles, then 200 ml of ethanol alcohol (%99) is added for 24 hours by soxhlets apparatus . New products were concentrated with rotary evaporator apparatus . It is dissolved in 5 ml of ethanol, adding 30 ml. Sulfuric acid (2%), then using rotary evaporator apparatus to remove ethanol alcohol. Mayer assay gives white product to ensure presence of alkaloids. Hydroxide ammonium (%10) was putt in separating funnel putting (10) ml from chloroform . Mixing of product was separating into two layers, selecting the bottom layer because it contains alkaloids. It was concentrated with rotary evaporator, a new dry product kept in icebox (Samaraei,1983).

3.7.2. Extraction of Crude Phenolics

Method of (Ribereaun – Gayon ,(1972) was used to extracts phenolics, 20 gm of dried extract put in a glass flask with 400 ml. of (%2) acetic acid by using the reflex condenser in (70) degree centigrade water bath for 8 hours . New suspension put with N-propanol and sodium chloride substances in the Suppression of separation been taking the top layer containing phenolic substances, then it was focused with evaporator rotor and product was dried and kept in the fridge.

3.7.3. Extraction of Crude Terpenoids

The Method of Harborne (1984) has been followed in preparing crude terpenoids compounds extract, that weighs 20 g. of dry matter powder then extracted with chloroform solvent by soxholet apparatus by using 200ml of chloroform solvents at 45c^{o} temperature for 24 hour. Then, the sample was extracted and focus by rotary evaporators. The sample containing the terpenoids compounds extract was dried up in the oven to 45-40 c ^{o} degree . The dry material was preserved in glass bottles until use. Reagents were conducted using standard procedures to detect the presence of alkaloids, phenolics and terpenoids substances.

Due to the efficiency of terpenoids compounds , they were chosen to extract effective compounds in it but for difficulty to collect a quantity from it because it contain a high percentage of volatile oils therefor an essential oils are chosen for GC/MS analysis .

3.8. Crude Secondary Compounds Reagents

3.8.1. Crude Alkaloid Compounds Reagents

3.8.1.1. Mayer's Reagent

Used to identify alkaloids, it is prepared with 13.5 g. of mercury chloride and 50 g. of potassium iodide in a liter of distilled water in an acidic environment. 2-1 ml is added to 5 ml. of extract, a white deposit appears to brown for the alcohol extract, while the water extract did not show deposit and the test is carried out using an hour bottle (Antherden, 1969; Harborne, 1984).

3.8.1.2: Tannic Acid Reagent

This detector is used to deposit alkaloids, which is prepared from 1% tannic acid and added to it (2-1) ml. to five ml. of water or alcohol extract and shows a white tanned turbidity (Alrubei, 1999).

3.8.1.3: Dragendroff s Reagent

Their reagent reveals all alkaloids and brings with the addition of 0.6 g. of psmouth nitrate and two ml. of concentrated hydrochloric acid to 10 ml. distilled water (solution 1), and prepare the solution (2) by adding six g. of potassium iodide to 10 ml. distilled water and the solution (1) and (2) was mixed and added to the mixture seven ml. of concentrated hydrochloric acid, mixes the sweeteners and adds 1-2 ml. of it to five ml. of water or alcohol extract, orange or reddish orange color shows the evidence of the presence of alkaloids (Harborne, 1973).

3.8.2: Crude Phenolic Compound Reagents

3.8.2.1: Lead Acetate (1%) Reagent

It is water or alcoholic solution for 1% lead acetate. The amount of the detector is added to an equal amount of alcohol extract, showing a yellow deposit evidence of the presence of tannin compounds (Alrubaei, 1999).

3.8.2.2. Iron Chloride (1%) Reagent

This detector is used to determine the presence of simple tannin and phenolics and this detector prepares from dissolving 1% of the iron chloride in distilled water by 1%, giving this detector a green or blue color when added to the amount of extract found in the bottle of an hour indicating the presence of phenolic compounds (Harborne, 1984).

3.8.2.3. Sodium Hydroxide Reagents NaOH

Coumarins and Phenyl propanoids of phenolic are detected by adding 5% sodium hydroxide water solution on five ml. of the plant's alcohol extract by using the filtration sheets in test tube that found in a boiling water bath for several minutes until the extract boils, a bluish green color appears when exposing the paper to ultra-violate radiation for 5-10 minutes (Harborne, 1984).

3.8.3. Terpinoids Reagents

3.8.3.1. Foam Reagent

It is used to detect the presence of saponins in terpenoid compounds. If the bottle containing a quantity of water extract was shaken, a dense foam appears on the surface of the extract and remains for a long time as evidence of the presence of terpinoids (Harborne, 1984).

3.8.3.2. Mercury Chloride (Hgcl2) Reagent

It is used to detect saponin from terpinoids and prepared from the addition of (1-2ml) from (1%) of mercury chloride to (5 ml.) of water extract, appear white deposits (Harborne, 1984).

3.9. Collection of Live Adults *E. vermicularis* Worm Samples

The live adult worms were collected directly from the anues of children at midnight. Then they were washed with distal water and the parasites were kept in petridishes with phosphate buffer saline PBS in 37 c° in incubator until the *In vitro* evaluation was started (Al- Madhi, 2016; Zenebe *et al*.,2017). The samples of helminths were collected from patients and diagnosed by light microscope using direct wet Smear for adult pinworms.

3.10.The Effects of Secondary Compound Extracts on Adult *E. vermicularis* Worm *In Vitro*

Preparation of stock solutions of secondary compound extracts of the *C. limon* plant fruits which prepare by the melt (2) g. of dry extract in (5) ml. of distal water . Therefore , stock solution become (400) mg. /ml. stock solutions are used for concentrations prepared (100,200 and 300mg. /ml.) control is prepared from phosphate buffer saline PBS only . Four groups of worms, each group with five worms one of them represented a control group , each group have been treated with an desired concentration of extracts and albendazole drug . The worms were assembled in petridishes which contain one ml of PBS then put in incubator , after one hour, one ml. of each concentrations were add to a petridishes that contain parasite each on its own for three replicates (Al- Madhi, 2016). The amount of time it took for all three subjects to become completely paralysed and pass away was noted (Al- Madhi, 2016; Khatiwora, 2018).

3.11.The Effects of Albendazole with Milligram Concentration on Adult *E. vermicularis In Vitro*

Albendazole drug was purchased from local pharmacy in Babylon province, its present as tablet with 400mg. the stock solution was prepared by adding 400mg. of drug powder with one ml. of distal water to become 400mg./ml. which mix by blender and a concentrations (100, 200 and 300 mg./ml.) were prepared from it in addition to control group , then from each concentrations take one ml and added to a petridishes that worm found each on its own.

3.12. Extraction of Essential Oils

The essential oil was obtained using the process of hydrodistillation, and the fruits were rinsed thoroughly with cold water. Following the addition of three liters of distilled water, a total of 500 g. of fruits was placed inside of a clevenger device . The clevenger apparatus was heated to a temperature of boiling water for a period of six hours, during which time the essential oil of distilled fruits was collected in a dry glass vial. There were three separate runs of the hydrodistillation. The essential oil of the fruits that was obtained was processed with anhydrous sodium sulphate, which eliminates any leftover moisture, and then it was placed in a tube, sealed, and chilled to 4 Celsius degrees for further examination (Paw *et al*., 2020).

3.13. Chemical Analysis of Essential Oils Using GC/MS

Gas chromatography and mass spectrometry (GC/MS) was used in order to do an analysis on the essential oil of fruits. An agilent technology (Little Falls) 6890 series gas chromatography (GC) system that was outfitted with a 5973 (MS) detector and a 7683 series auto-injector was utilized for the GC/MS analysis of the essential oil of *C. limon* plant fruits. Compounds were separated using a Rtx®-Wax capillary column (with dimensions of 30 meters by 0.25 millimeters and a film thickness of 0.25 micrometers . As the carrier gas, helium of the grade 5N5 was used, and the flow rate was set at 0.8 ml./minute while the split ratio was set at 60:1.

The volume of the sample injection was 1 microliter, and the temperature of the injector was 230 C°. After maintaining a temperature of 70 C°for two minutes in the column oven, the temperature was set to increase gradually to 130 C° at a rate of thirty c° per minute before the gradient was altered to 230 C° at a rate of ten c° per minute. At the end, the temperature was maintained at 230 C° for the whole run period of twenty minutes. For the purpose of detection, an electron ionization (EI) device with an ionization energy of 70 electron volte was used.

The temperature of the ion source was set to 230 C°, the temperature of the interface was set to 250 C° , and the voltage of the detector was set to 2 kilovolts.

The mass spectrum was obtained by scanning the mass range from 20 to 800 amu at a rate of 0.98 scans per second while in scan mode. The measurement was carried out twice for each sample, with a two-minute interval of solvent delay between each set (Wu *et al*., 2014).

For a difficulty of extracting a concentrated of each compound in *C.limon* fruits extract because its needed to a high cost and time, and since a limonene compound, it's a most concentrated in *C.limon* fruits extract according to researchers such as Shakir and Salih (2015),Al-Jabri and Hossain (2016), Kaskooz (2019) and Paw *et al.* (2020) who analyzed essential oils using GC-MS technical where they found a main bioactive compounds with high content in *C.limon* fruits was limonene compound, So it was chosen to test its effectiveness on *E. vermicularis* worm *In Vitro*.

3.14.The Effects of D-limonene Compound on Adult *E. vermicularis* Worm *In Vitro* .

D-limonene compound is liquid with 0.86g/ml. concentration. The stock solution was prepared by adding 1ml. from D-limonene compound with adding 0.1% of Tween 80 (polysorbate 80) that used as a solvent and volume complemented to 1L of distal water therefor a stock solution become 860µ/ml and from it a concentrations (25, 50, 100, 200 and 400 µg/ml) were prepared , then taken one ml of each concentrations and have been added to a petridishes that contain parasite each on its own (Shah and Mehta, 2018).

3.15.The Effects of Albendazole Drug with Microgram Concentration on Adult *E. vermicularis* Worm *In Vitro*

The stock solution of albendazole drug was prepared by adding 200mg. of this drug with 20ml. of distal water to become 20mg/ml or 20000µ/ml and a concentrations (25, 50, 100, 200 and 400 µg/ml) were prepared in addition to control group, the time of paralysis and death was record with three replicates then

taken one ml of each concentrations and added to a petridishes that included worm each on its own for albendazole drug (Shah and Mehta, 2018).

3.16. Estimation of Pinworms Worm Viability *In Vitro*

The movement and death of worms has been monitored by looking, no movement or death of worm was identified (Al- Madhi, 2016).

3.17. Molecular Study

3.17.1. Kits

There are two kits that used for extraction of DNA from stool sample as mention in table 1.

Table (3-3) The used kits with their companies and origin.

No.	Kit	Company	Country
1	**Presto™ Stool DNA Extraction Kit**	**Geneaid**	**Taiwan**
	Stool Lysis buffer (ST1)		
	Binding buffer (ST3)		
	Washing buffer		
	Elution buffer		
	GD column		
	Collection tube 2ml		
2	**GoTaq Green PCR Master Mix**	**Promega**	**USA**
	Tap DNA polymerase dNTPs (dATP, dCTP, dGTP, dTTP) Tris-HCl pH 9.0, KCl, $MgCl_2$ Stabilizer and Tracking dye		

3.17.2.Primers:

The primers are used for detection *E. vermicularis* based on mitochondrial cytochrome oxidase subunit one (Cox1 gene) were designed by NCBI website (AP017684.1) and primer 3 plus. The primers were provided from Scientific Resercher.Co.Ltd, Iraq as following table:

Table (3-4): The primers used for detection worm based on Cox1 gene.

Primers	Sequence 5'-3'		Product size
Cox1 gene Enterobius vermicularis	F	TGTGTTGGCTGGGGCTTTAA	407bp
	R	GCTGCACAACTAAACGTCCC	

3.18. Polymerase Chain Reaction

PCR technique was performed for detection *E. vermicularis* based on mitochondrial cytochrome oxidase subunit one (Cox1 gene from stool samples. These techniques are done based on (Al-Samaraei, 2020), as following:

3.18.1. DNA Extraction

Extracted DNA was done by kit called Presto™ Stool DNA, as following steps:

A- Sample Lysis Step:

1- A 250µl of the stool sample was moved into bead beating tube, and then 800µl ST1 buffer and vortex briefly then put in an incubator at 70 Cº for 5 minutes. By using of the vortex at high speed for ten min. at 25 Cº, then centrifuged for two minutes at 8,000xg.

2- The supernatant (500) µl was transferred to 1.5 ml of microcentrifuge tube.

C. DNA Binding Step:

1- An 800 µl ST3 buffer was poured into the flow-through, and immediate mixing ensued in the form of violent shaking for a duration of 5 seconds. After that, a GD Column, which was represented by a green ring, was positioned inside of a 2 ml Collection Tube**.**

2- A sample combination that was 700 microliters in volume was poured into the GD Column. After that, the mixture was centrifuged for one minute at 16000 x g at room temperature, and the flow-through was discarded**.**

3- Place the GD Column into the 2 ml Collection Tube. After that, the remainder of the sample combination was moved into the GD Column, for one minute at 25 Cº was centrifuged at a speed of 16,000 x g**.**

4- The flow – through was discarded, insertion of the GD Column in tube (2) ml .

D. Wash Step:

1- 400 microliters of ST3 buffer were poured into the GD Column. After that, centrifuging of the mixture for half minute at (16000) x g at (25) Cº**.**

2- The flow-through was left, and the GD Column was reinserted into the 2 ml collection tube once it had been cleaned**.**

3- A wash buffer volume of 600 microliters was added to the GD column. After that, the mixture was centrifuged for 30 seconds at 16,000 x g at room temperature

4- The flow-through was discarded, and the GD Column was reinserted into the 2 ml collection tube once it had been cleaned**.**

5- The column matrix was dried by centrifuging it at 16,000 x g for three minutes at room temperature. This was done while the GD column collecting tube was empty.

E. Elution Step:

1- A fresh micro centrifuge tube measured 1.5 millilitres was inserted into which the dry GD Column was introduced. After that, 100 microliters of a heated elution buffer**.**

2- After allowing the GD Column to left 120 seconds, the buffer of the elution was given the chance to be entirely absorbed. After that, the DNA that had been purified was extracted by centrifuging it for two minutes at a speed of 16,000 x g at room temperature.

3.18.2. The Purity and Concentration of DNA

Extraction of DNA from the samples was done by the spectrophotometer (nanodrop) . Nanodrop measures the DNA purity at the absorbance (260-280) nm**.**

1. Launch the nanodrop programme, then choose the relevant application from the menu (Nucleic acid, DNA)
2. A dry wipe was used to thoroughly clean each of the measuring pedestals on many occasions. Then, using a pipette, gently transfer of without enzymes water over the bottom measuring pedestals to blank the system**.**
3. After the sample arm was lowered, the OK button was hit in order to initiate the nanodrop process. Following that, the pedestals were cleaned, and put put to the measurement. Finally, the results were recorded.

3.18.3. Preparation of the Master Mix:

The PCR master mix was created by utilizing (Taq Green PCR Master Mix), and the master mix was done based on company indirections as follows.

Table (3.5)Standard PCR master mix components

PCR Master mix	Volume
DNA template	5µL
Cox1 gene Forward primer (10pmol)	2µL
Cox1 gene Reverse primer (10pmol)	2µL
Go taq Green Master mix	12.5µL
PCR water	3.5 µL
Total volume	25µL

After that, these PCR master mix component that mentioned in table above transferred into Exispin vortex centrifuge at 3000rpm for 3 minutes. Then placed in PCR Thermocycler.

3.18.4. Conditions of the Thermocycler:

Conventional PCR thermocycler conditions was performed using the following conditions as table( )

Table (3.6) PCR thermocycler conditions

PCR step	Temp.	Time	repeat
Initial Denaturation	95ºC	5min.	1
Denaturation	95 ºC	30sec.	35 cycle
Annealing	58 ºC	30sec	
Extension	72 ºC	2min.	
Final extension	72 ºC	5min.	1
Hold	4 ºC	Forever	-

3.18.5. Analysis of the PCR Product:

By agarose gel electrophoresis, the PCR products was analyzed as following steps:

1- The 1.5 % agarose gel was made by utilizing100 ml of 1X TBE and digesting it in a microwave at 100 C° for fifteen min. After that, then cooled to 50 C°.
2- Adding of the three microliters of a ethidium bromide stain to the agarose gel.
3- Pouring of the agarose gel in the tray after the comb is placed in the correct orientation, then left at 25 C° for fifteen minutes, the comb was carefully removed from the tray.

4- The gel tray was positioned inside the electrophoresis chamber and then filled with 1X TBE buffer**.**

5- 10 µL from PCR product and 3 µl of (100bp Ladder) were individually added to the wells of the comb, starting with the first well**.**

6- The electric current of (100) volts and (80) Am for (60) minutes**.**

7- PCR products were seen by UV Transilluminator .

3.19: DNA Sequence Method

The DNA sequencing method was carried out for study detection of *E.vermicularis* by using mitochondrial cytochrome oxidase subunit one Cox1 gene according to NCBI BLAST *E. vermicularis* isolates. The PCR products were sent to Macrogen Company by DHL for make the sequencing by sequencing system.

The DNA sequencing was done by (Mega V: 6.0) software and many sequence analysis of Cox1 genes based ClustalW alignment analysis and the evolutionary distances were computed by using UPGMA method.

3.20: Statistical Analysis:

Analyze of data of epidemiology by Chi-Square (X^2) while plant extract study carry out by using factorial experiments with completely randomized design(C.R.D) and using least significance differences(L.S.D.) at level ($P \leq 0.05$) by using the statistics system (SPSS) depending on (Al-Rawi and Khalaf-Allah , 2000).

4-Results.

4.1. Parasitological Study Results

4.1.1. Prevalence of *Enterobius vermicularis* Infections:

Sixty eight point thirty one (68.31%) from ninty hundred seventy two (972) children were found to be infected with *E. vermicularis* as shown in table (4.1) and appendix (9).

Table(4.1) Percentage of infected children with *E. vermicularis* using a lugol's iodine stain and scotch tape method.

Parasite	Total sample	Positive		Negative	
		No.	%	No.	%
E. vermicularis	972	664	68.31	308	31.68

4.1.2. Distribution of *Enterobius vermicularis* Infection Among Children According to the Gender

The current study show that no significance relationship between a two genders. The male children have a high percentages o f infections were (55.42%) while the infected female children have a less percentages were (44.57%), as shown in table (4.2) and appendix (11)

Table (4.2) Numbers and percentages of patients infected with *E. vermicularis* according to the gender.

Gender	Examined samples	Infected samples		Non infected samples		χ2	Significance
		N	%	N	%		
Male	543	368	55.42	175	56.81	0.166	0.729
Female	429	296	44.57	133	43.18		
total	972	664	100	308	100		

4.1.3.Distribution of *Enterobius vermicularis* Infection Among Children According to the Residence.

Table (4.3) and appendix (12) appears that urban areas have a high percentage of infection which were (74.21%) while rural regions have a high percentage of infection which were (25.45%) with a significance differences.

Table (4.3)Distribution of *E. vermicularis* infection among children according to the residence.

Residence	Examined	Infected		Non infected		χ^2	Significance
		N	%	N	%		
Urban	667	495	74.21	172	55.84	34.18	0.0001
Rural	305	169	25.45	136	44.15		
total	972	664	100	308	100		

4.1.4.Relationship Between the Age Groups and the Infection with *Enterobius vermicularis*.

The results in table (4.4) and appendix (13) shows that a significance relationship between an age groups and an infection where an age group (8-10) years has a highest percentage of infected children with *E. vermicularis* worm that was (53.31%), while a lowest percentage that was present in (2-4) age group which was (4.36%).

Table(4.4)Relationship between the age groups and the infection with *E. vermicularis* among children.

Age groups in years	Examined	Infected		Non infected		χ2	Signifi cance
		N	%	N	%		
2-4	68	29	4.36	39	12.66	62.26	0.0001
5-7	130	74	11.14	56	18.18		
8-10	442	354	53.31	88	28.57		
11-13and over	332	207	31.17	125	40.58		
Total	972	664	%100	308	%100		

4.2. Pharmacological study

4.2.1.Effect of Cold and Boiling Water Extract of *Citrus limon* Plant Fruits and Date Vinegar on Paralysis and Die Worm in Different Concentration *In Vitro*.

Table (4.5) appear a high effects of cold aqueous extract of *C. limon* plant fruits on paralysis and die of worms especially by 300 mg/ml concentration where its led to paralysis and die of worms at 29.00 and 57.33 minute , respectively, while a concentrations of 200 and 100 mg/ml led to paralysis at 43.00 and 46.00 minute and for die of parasite at 62.33 and73.67 minute with control 1400.67 and 1542.00 minutes, respectively, also the hot aqueous extract of *Citrus limon* plant fruits has effect on paralysis and die of worms in 300 mg/ml concentration where its led to paralysis and die of worms at 67.33 and 104.00 minute . As to hot aqueous extract of date vinegar has a lowest effect on parasite where its led to paralysis and die of worms at 139.00 and 246.00 minute in 300 mg/ml concentration while 200 and 100 concentrations led to paralysis at 189.00 and 269.00 minute and for die of worms 301.33 and 424.33 minute with control 1423.33 and 1525.67 minutes, respectively, with significance differences.

Table(4.5) The Effect of Overlapping Concentration Extracted for Cold and

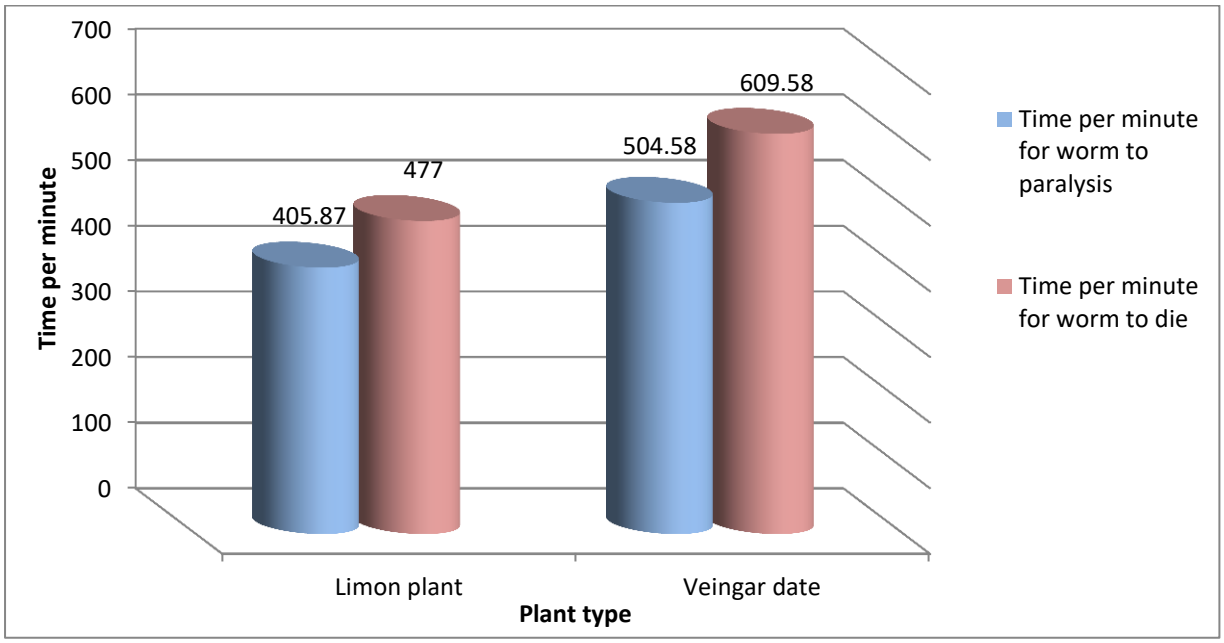
Boiling Water for *Citrus limon* Plant and Date Vinegar on Worm Paralysis and Die *In vitro*.

	Extract type	concentration mg/ml	Time per minute for worm to paralysis		Time per minute for worm to die	
			Mean	±S.D.	Mean	±S.D.
Citrus limon plants	cold extract	300	29.00	.6	57.33	.3
		200	43.00	.6	62.33	.9
		100	46.00	.6	73.67	.3
		Control	1400.67	19.7	1542.00	21.1
	Hot extract	300	67.33	1.2	104.00	1.0
		200	111.67	1. 5	171.33	1.3
		100	146.00	.6	245.33	.3
		Control	1403.33	18.3	1560.00	34.6
Date Vinegar						
	cold extract	300	110.00	.6	193.00	5.5
		200	141.00	.6	255.00	.57
		100	342.33	1.2	389.00	12.5
		Control	1423.00	21.7	154.33	17.6
	Hot extract	300	139.00	.6	246.00	.57
		200	189.00	.6	301.33	.33
		100	269.00	.6	424.33	1.2
		Control	1423.33	20.3	1525.67	17.1
LSD at probality level 0.05			28.95		35.65	

4.2.2.Effect of Plant Type Factor for Aqueous extracts on Worm to Paralysis and Die *In Vitro*.

Static analysis demonstrates that *C. limon* plant fruits extracts possess a great activity more than aqueous extracts of date vinegar where its average arithmetic for paralysis and die of worms equal405.87and477.00 minute, respectively, comparative with date vinegar extracts that have less effect which was 504.58 and 609.58 minute, respectively, with clear significance differences as shown in figure (4.1) and appendix

(14).

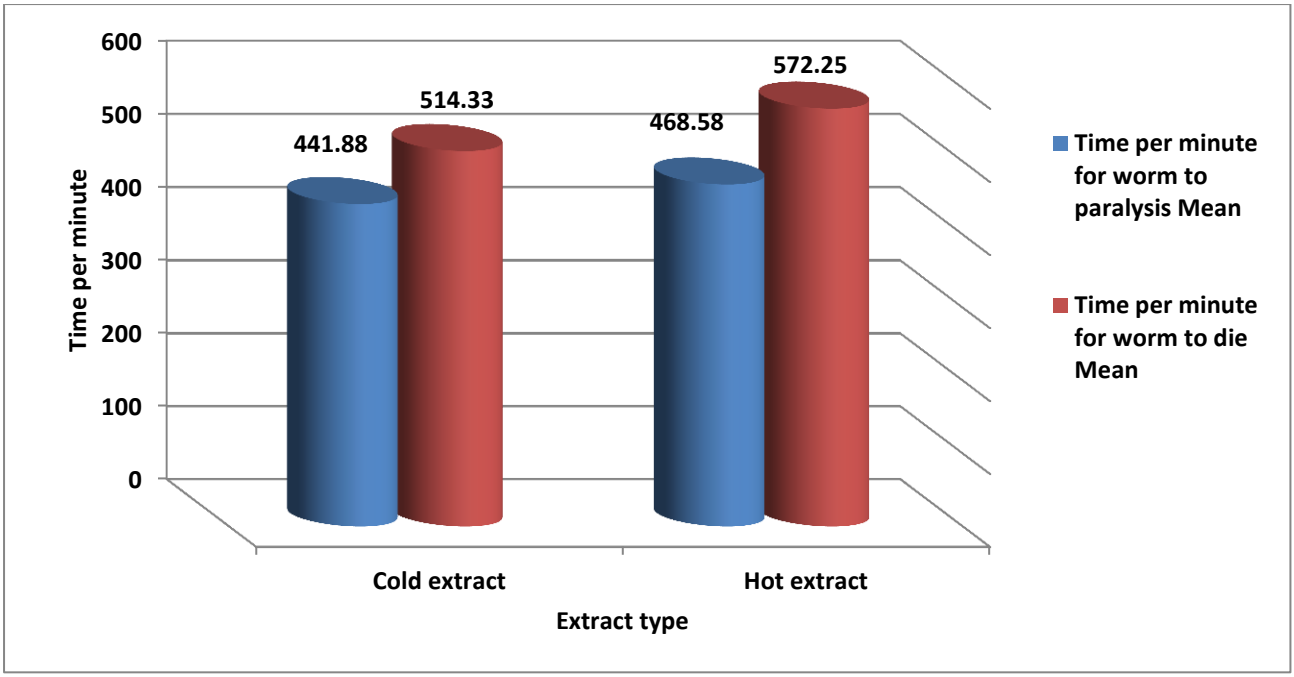


LSD at probality level 0.05 =10.23 for paralysis and =12.60 for death

Figure(4.1)The effect of plant type factor of water extracts of *Citrus limon* plant fruits and Vinegar date on worm paralysis and die *In vitro*.

4.2.3.Effect of Extract Type Factor for Aqueous extracts on Worm to Paralysis and Die *In Vitro*.

The figure (4.2) and appendix (15) shows that cold aqueous extract of both *C. limon* plant fruits and date vinegar has a high effect on worm to paralysis and die where its average arithmetic for paralysis and die were 441.88 and 514.33 minute, respectively, while hot aqueous extract has less effect where it's average arithmetic were 468.58 and 572.25 minute, for paralysis and die of parasite with significance differences.

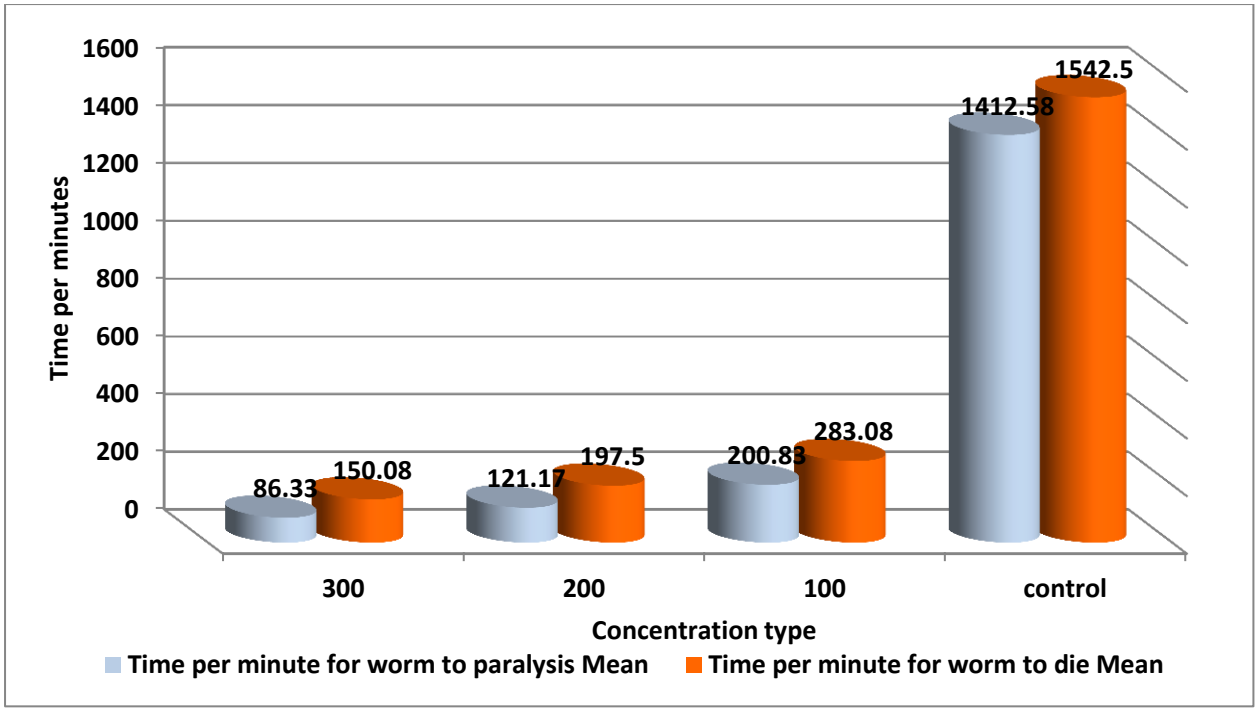


LSD at probality level 0.05 =10.23 for paralysis and =12.60 for death

Figure (4.2)The effect of extract type factor of aqueous extracts of *Citrus limon* plant fruits and date vinegar on worm to paralysis and die *In vitro*.

4.2.4.The Effect of Concentration Type Factor Aqueous extracts on Worm to Die and Paralysis *InVitro*.

Figure (4.3) and appendix (16) appears that effect of concentration type factor on worm to die and paralysis where 300 mg/ml concentration possess a great effect from other concentrations on worm to die and paralysis with significance differences , where its average arithmetic for paralysis and die were 86.33 and 150.08 minute , respectively, compare with a little effect concentration which is 100 mg/ml where its average arithmetic for paralysis and die were 200.83 and 283.08 minute with control (1412.58) for paralysis and (1542.50) for die, respectively.

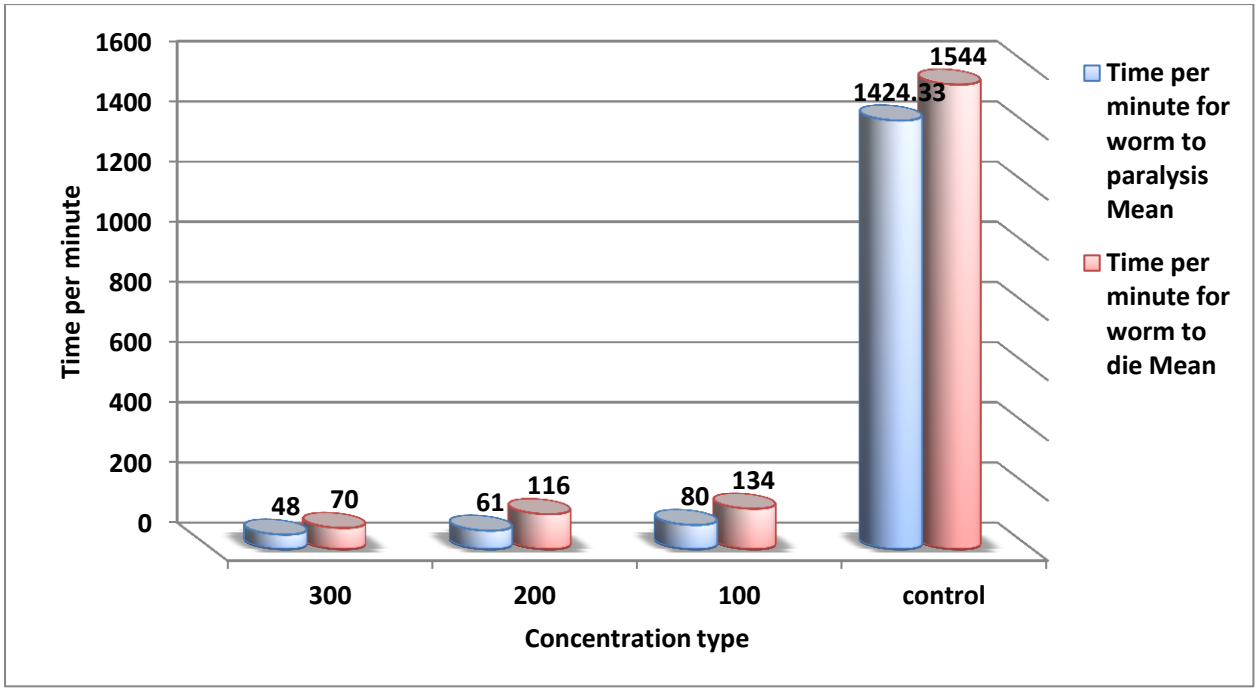


LSD at probality level 0.05 =14.47 for paralysis and =17.82 for death

Figure (4.3)The effect of concentration type factor for aqueous extracts of *Citrus limon* plant fruits and date vinegar on worm to die and paralysis *In vitro*.

4.2.5.The Effect of Albendazole Drug on Worms *In Vitro*.

The albendazole drug was very efficient in paralyzing and killing worms especially in 300 mg/ml concentration where its led to paralysis at average arithmetic 48.00 minute and to killing at average arithmetic 70.00 minute, with significance differences, while a less effect concentration was 100 mg/ml concentration was 80.00 minute for paralysis and 134.00 minute for death with control 1424.33 and 1544.00 minutes for paralysis and die ,respectively, with significance differences as shows in figure (4.4) and appendix (17).



LSD at probality level 0.05 =34.55 for paralysis and =34.25 for death

Figure(4.4)The effect of Albendazole drug concentrations on worm paralysis and death *In vitro*.

4.2.6. The Effect of Concentrations Interference of Secondary Compounds for *C. limon* Plants on Worm Paralysis and Death *In Vitro*.

Table (4.6) appear a high effects of terpenoid compounds extract of *Citrus limon* plant fruits on paralysis and die of worms especially with 300 mg/ml concentration where its led to paralysis and die of worms at 18.00 and 33.00 minute, respectively, while 200 and 100 mg/ml concentrations that led to paralysis and die of worms at 27.00 and 46.67 minute for 200 mg/ml concentrations and 38.00 and 65.00 minute, respectively for 100 mg/ml concentrations with control 1386.33 and 1560.00 minutes, respectively, followed by phenolic compounds that led to paralysis and die of worms with 300 mg/ml concentration at 32.33 and 53.00 minute, respectively, as 200 mg/ml concentrations led to paralysis at 38.67 minute and to die at 64.00 minute, also 100 mg/ml concentrations led to paralysis at 70.00 minute and to die at 106.00 minute, with control 1424.33 and 1551.67 minutes for paralysis and die ,respectively, followed by alkaloid compounds that led to paralysis and die of worms with 300 mg/ml

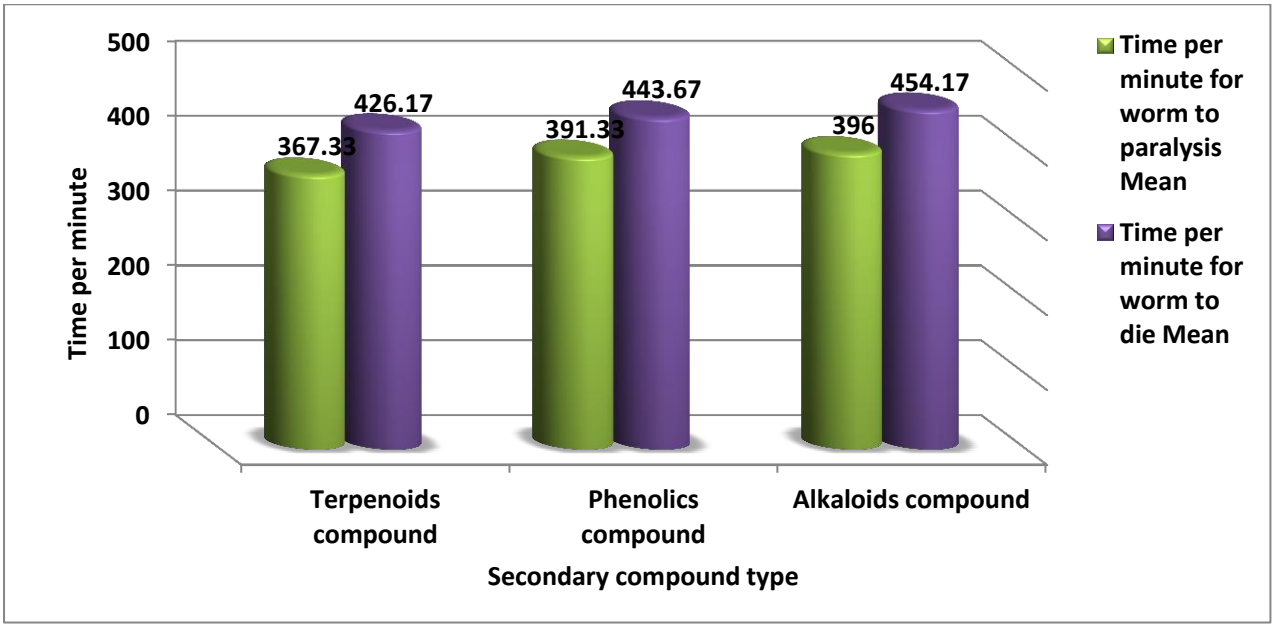
concentration at 40.00 and 63.00 minute , respectively, also 200 mg/ml concentrations led to paralysis at 62.00 minute and to die at 95.00 minute, also 100 mg/ml concentrations led to paralysis at 75.00 minute and to die at 118.67 minute with control 1407.00 for paralysis and 1540.00 minutes for die .

Table (4.6)The Interference Effect of Secondary Compound Concentrations for *C. limon* Plants on Worm Paralysis and Death *In vitro*.

Secondary compound type	concentration mg/ml	Time per minute for worm to paralysis Mean	±S.D.	Time per minute for worm to die Mean	±S.D.
Terpenoids compounds	300	18.00	.58	33.00	.58
	200	27.00	.58	46.67	.88
	100	38.00	.58	65.00	.58
	Control	1386.33	1.45	1560.00	15.28
Phenolics compounds	300	32.33	.88	53.00	.58
	200	38.67	.33	64.00	.58
	100	70.00	.58	106.00	.58
	Control	1424.33	21.17	1551.67	21.28
Alkaloids compounds	300	40.00	.58	63.00	.58
	200	62.00	.58	95.00	.58
	100	75.00	.58	118.67	.33
	Control	1407.00	20.50	1540.00	20.00
LSD at probality level 0.05		24.90		26.94	

4.2.7.Secondary Compound Type Effect for *Citrus limon* Plants on Worm *In Vitro*.

Terpenoids compounds was great efficiency in paralysis and die for worm where its average arithmetic for paralysis and die was 367.33 and 426.17 minute, respectively , whereas a least influential secondary compound was for alkaloids compounds where its average arithmetic for paralysis 396.00 and for death was 454.17 minute, respectively, with significance differences as shows in figure (4.5) and appendix (18).

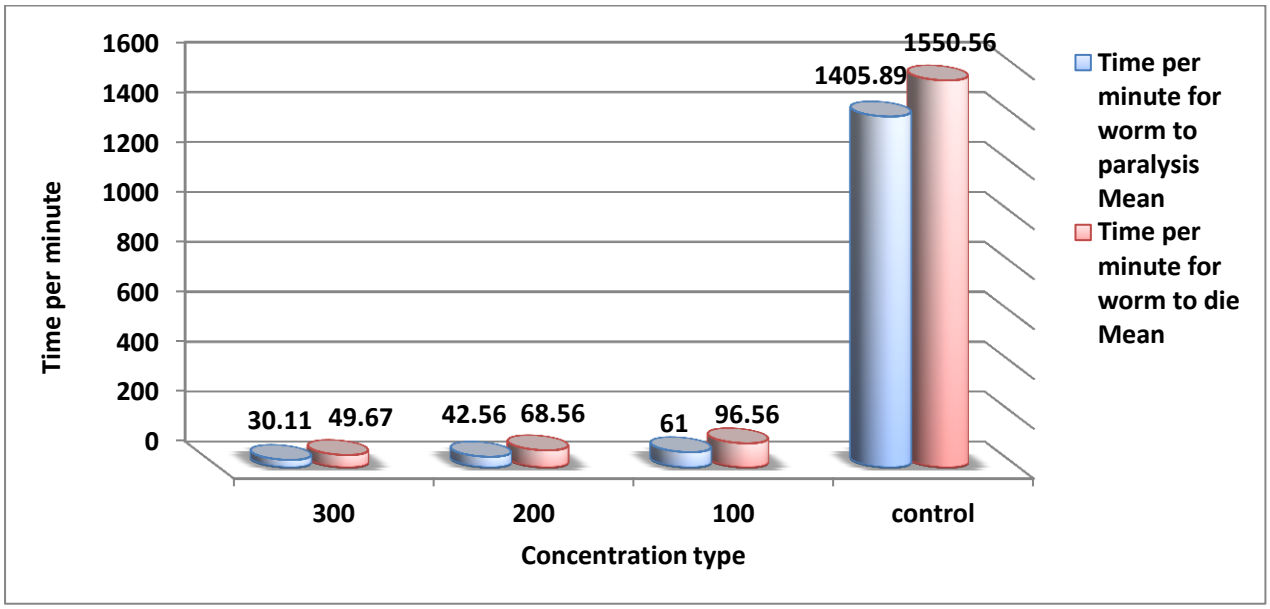


LSD at probality level 0.05 =12.45 for paralysis and =13.47 for death

Figure (4.5) Secondary compound type factor effect for *C. limon* plants on paralysis and die for worm.

4.2.8.The Effect of Concentration Type for Secondary Compound of *Citrus limon* Plants Fruits on Worm *In Vitro*.

Figure (4.6) and appendix (19) appears that concentration type factor was effects on worm at 300 mg/ml concentration with a great efficient where its average arithmetic for paralysis and die were 30.11and 49.67 minute , respectively, whereas a little effect concentration which is 100 mg/ml concentration where its average arithmetic were 61.00 and 96.56 minute with control 1405.89 and 1550.56 minutes for paralysis and die, respectively, with significance differences.



LSD at probality level 0.05 =14.38 for paralysis and =15.55 for death

Figure (4.6)Concentration type effect factor for Secondary compound for *C. limon* plants fruits on paralysis and die for worm.

4.2.9.GC-MS Analysis of Bioactive Substances of *Citrus limon* Plant Fruits.

GC-MS technical analysis of essentials oil of Iraqi *C. limon* plant fruits revealed a presence of seventeen compounds where appear a separation when start analysis of crude phenolic compound extracts sample at retention time were 9.260, 13.500, 14.386, 17.651, 23.251, 27.194, 46.798, 53.936, 54.742, 55.999, 56.559, 60.006, 60.320, 60.480, 61.406, 61.618, 62.263 minutes, where as an area of these compounds were 8456,18585, 17080, 48457, 61188, 32907, 155417,428115, 434884,387914 , 421121, 467189, 473928, 467308, 467189, 473919 and 480956 mAu (mill Area unit), respectively, as shown in figure (4-7) which are arrangement in table (4-7).

From these results we notes that limonene compound is present in crude phenolic compound extracts sample of Iraqi *C. limon* plant fruits at retention time 17.651 minute which is close to retention time of stander limonene compound in library search report of GC-MS apparatus which is17.50 minute as shown in figure (4-8).

Table (4.7)GC-MS analysis of *C. limon* plant fruits.

Peak Number	compound Name	Retention Time	% Peak Area
1	2-furan-carboxaldehyde	9.260	0.71
2	2,5-Furandione, 3-methyl	13.500	4.93
3	2-Furancarboxaldehyde, 5-methyl	14.386	1.20
4	D-Limonene	17.651	0.71
5	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	23.251	0.83
6	2-Furaldehyde, 5-(hydroxymethyl)	27.194	6.15
7	5-(2-Thienyl)pentanoic acid	46.798	1.74
8	Nonadecane	53.936	0.30
9	Hexadecanoic acid, methyl ester	54.742	0.50
10	n-Hexadecanoic acid	55.999	11.66
11	9-Octadecenal, (Z)	56.559	1.75
12	9,12-Octadecadienoic acid	60.006	2.91
13	9-Octadecenoic acid	60.320	1.70
14	Cyclopropaneoctanal, 2-octyl	60.480	4.40
15	9,12-Octadecadienoic acid	61.406	13.27
16	9-Octadecenoic acid, (E)	61.618	40.43
17	1-Heptadecanecarboxylic acid	62.263	6.80

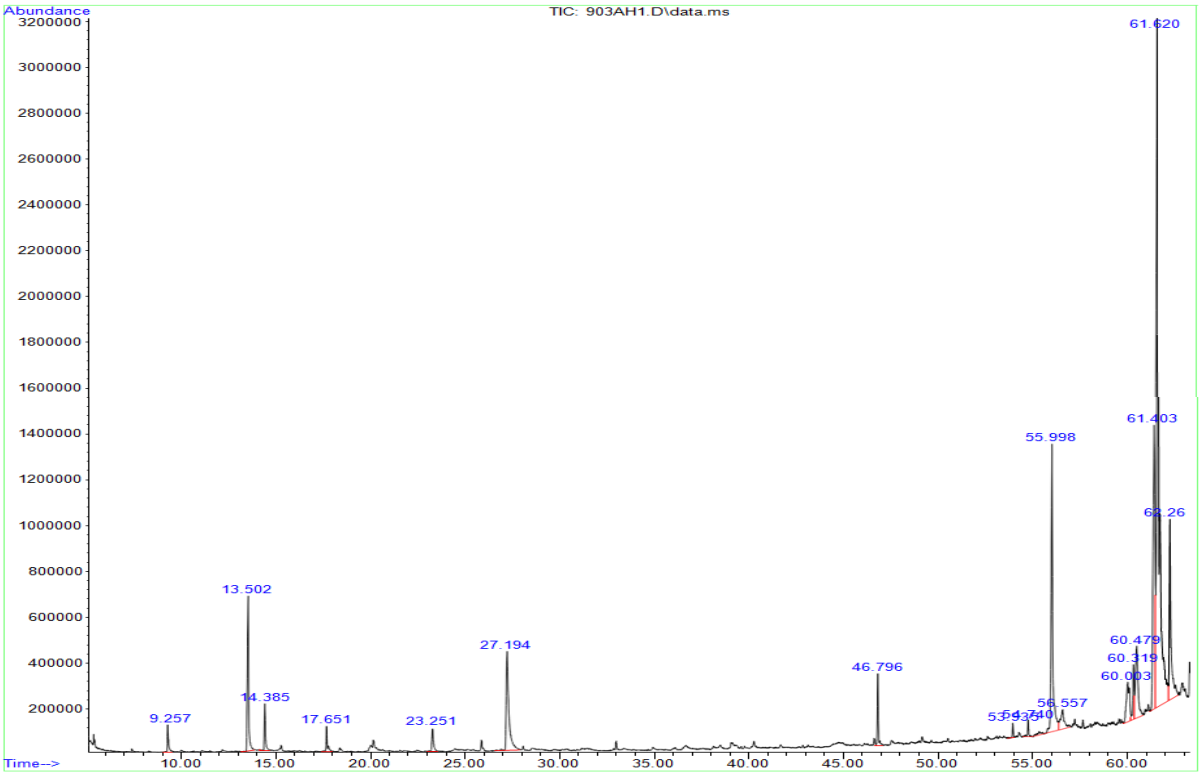


Figure (4.7) Chromatogram of the essentials oil of Iraqi *Citrus limon* plant fruits

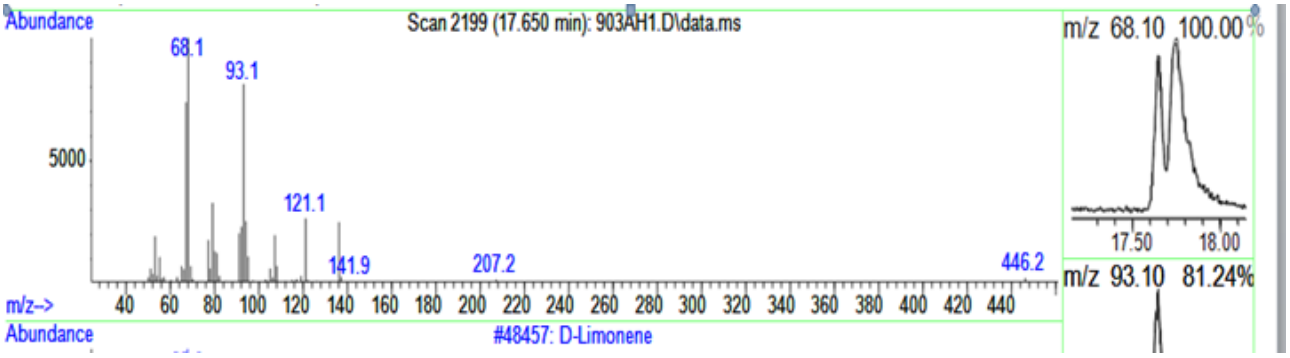


Figure (4.8)Chromatogram of the essentials oil of *Citrus limon* plant fruits in library search report of GC-MS apparatus.

4.2. 10.Limonene Compound Effects on Worms *In Vitro*

The static analysis of this study demonstrates that limonene compound possess a High efficient in 400 µ /ml concentration where its average arithmetic for paralysis was 10 minute and for die was 30 minute ,respectively, whereas a little effect concentration which is 25 µ /ml concentration where its average arithmetic for paralysis was 34 minute and for die was 66 minute for die with control 1554.6 and 1551.00 minutes for paralysis and die, respectively, with significance differences as shown in table (4.8).

Table(4.8)Limonene concentration effects on worm paralysis and die *In vitro* .

Concentration(µ /ml)	Time per minute for worm to paralysis		Time per minute for worm to die	
	Mean	±S.D.	Mean	±S.D.
400	10.00	1	30.00	1
200	13.00	1	36.00	1
100	15.00	1	41.00	1
50	20.00	1	52.00	1
25	34.00	1	66.00	1
control	1554.6	13.2	1551.00	1
LSD at probality level 0.05	2.84		1.77	

4.2.11.Albendazole Drug Concentrations Effects with µ/ml on Worm *In vitro* .

Table (4.9) appear that albendazole drug has a great effect on paralyzing and killing of worms especially in 400 µ /ml concentration where its led to paralysis at average arithmetic 145.00 minute and to killing at average arithmetic 243.00 minute with significance differences, while a less effect concentration was 25 µ /ml concentration where its average arithmetic was 322.00 minute for paralysis and 583.00 minute for death with control 1551.00 and 1553.33 minutes for paralysis and die, respectively, with significance differences.

Table (4.9) Albendazole Drug Concentrations Effects with µ/ml on Worm Paralysis and Die *In Vitro*

Concentration (µ /ml)	Time per minute for worm to paralysis		Time per minute for worm to die	
	Mean	±S.D.	Mean	±S.D.
400	145.00	1	243.00	1
200	177.00	1	301.00	1
100	192.00	1	382.00	1
50	235.00	1	465.00	1
25	322.00	1	583.00	1
control	1551.00	1	1553.33	1.5
LSD at probality level 0.05	**1.78**		**1.97**	

4.3.Molecular study Results

4.3.1.PCR Product for *COX*1 Gene

The Agarose gel electrophoresis results for PCR products in *E. vermicularis* worm showed appears *cox1 gene* bands at 407 bp PCR product size, as in Figure (4.9).

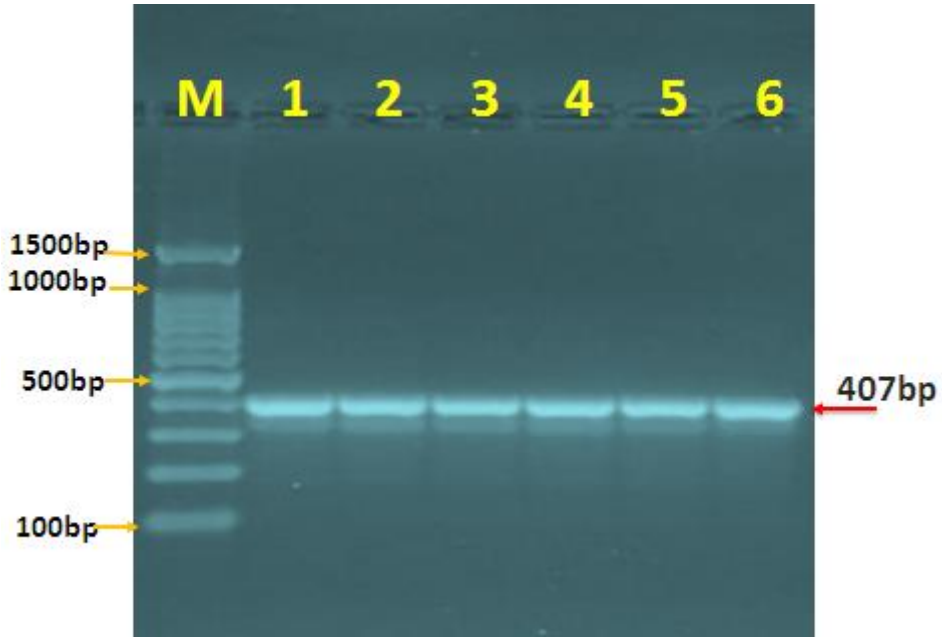


Figure (4.9) Electrophoresis photo illustrate PCR bands of *Cox1* gene in *E. vermicularis* from stool in Human. M: represents the ladder, (1-6) represents positive *E. vermicularis* with cox1 gene (407) bp.

4.3.2. Sequences of cox1 Gene.

Gene sequence was reading by sent the PCR products to Macrogen Company in Korea for performed gene sequencing by 407pb .

4.3.3. Cox1 Gene Sequence Analysis.

The *Cox1* gene sequencing analysis for local *E.vermicularis* isolate was conducted by Korea company and compared with NCBI-BLAST *E. vermicularis* related Genotypes isolates for determinate its identical with NCBI-BLAST global isolates as shown in table (4.10)

All these six local isolates for *E. vermicularis* were recorded in NCBI BLAST GenBank as a global scientific website (Table 4.10).

Table (4.10)Percentage of NCBI-BLAST Homology between our isolates and referenced *E. vermicularis*.

Isolate No.	Genbank accession no.	Homology sequence identity			
		Identical isolate	Country	Mutation %	Identity %
IQH-No.1	MZ505531.1	MH802611.1	Iran	1.1%	98.99%
IQH-No.2	MZ505532.1	MH802611.1	Iran	0.68%	99.32%
IQH-No.3	MZ505533.1	MH802611.1	Iran	0.68%	99.32%
IQH-No.4	MZ505534.1	MH802611.1	Iran	0.34%	99.66%
IQH-No.5	MZ505535.1	MH802611.1	Iran	0.68%	99.32%
IQH-No.6	MZ505536.1	MH802611.1	Iran	0.68%	99.32%

4.3.4.Phylogenetic Analysis.

The obtained alignments from CLUSTALW program of all sex sequences of Iraqi local isolates and their relationship with global isolate from GenBank was carried out using Maximum Composite Likelihood method MEGA software 6 version. The local *E. vermicularis* (IQH-No.1-IQH-No.6) isolates were showed genetic related to NCBI-BLAST *E. vermicularis* at entire genetic alteration (0.0080-0.0020) as shown in Figure (4.10).

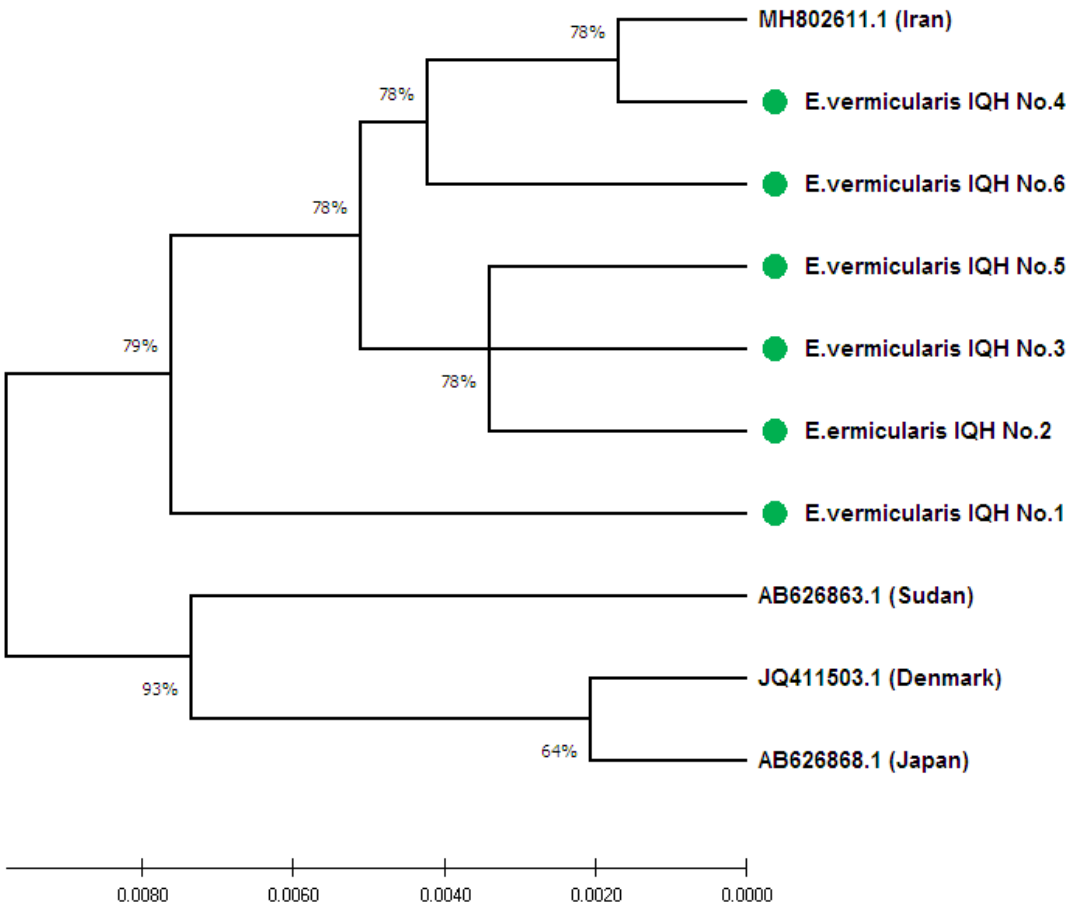


Figure (4.10). Phylogenetic tree depended on partial sequence of Cox1 gene which utilized for *E. vermicularis*. The tree was draw by computed Maximum Composite Likelihood method in (MEGA 6.0 software), our isolates of *E. vermicularis* showed related to NCBI- Iran isolate at entire genetic alteration (0.0080-0.0020).

5.Discussion

5.1.Parasitological study

5.1.1. *Enterobius vermicularis* Infections

E. vermicularis is a global helminthic parasite of humans that is especially common in temperate climates (Tomanakan *et al*., 2018), a worms may be visibly expelled with the stool therefor a worm identified macroscopically constitutes evidence of infection in the case of severe infection (wendt *et al*., 2019).

The results of this research or study shows that 664 children were infected with *E. vermicularis* parasite in Babylon province ,this result appear a high percent of infection among children and these findings (68.31) agree with results of Kadir and Amin (2011) 24.9%, Hama and Rahemo (2014) 29.8%, and close to the a results by Chai *et al*. (2015) 47.2%, Rahi and Morad (2017) 62%, Musa (2017) 78.48, also these findings disagree with a findings of Rhadi *et al*.(2018) 3.21%,

Salman *et al*.(2019) 0.6%, Alwaily *et al*. (2019) 34%, Hussein and Meerkhan(2019) 18.01%, Al-Ibrahhimi (2019) 43.67%, Al-Daoody and Al-Bazzaz (2020), 27.13%, Al-Saqur *et al*. (2020) 35.86 in all provinces of Iraq, Al-Samarai (2020) 7.80%, Ahmed *et al*. (2020) in Nineveh governorate, they were reported 13.26 % a percent of infection, as well as these findings less than findings of Hussein (2015) was that 83.9% and Hamarsheh (2021) was that 98.9.

The high incidence of parasitic infections among a children in Babylon province may be attributed to low socioeconomic service levels and sanitary conditions has not changed in recent years, frequent infections, parasitic resistances, the behaviors of fingers sucking and anal scratching were still risk factors for *E. vermicularis* infection (Chen *et al*.,2018), the infection transmission occur in the family by the siblings, as the human development index probably have direct effects on the prevalence of *E. vermicularis* (Chen *et al*., 2018; Taylor *et al*., 2018).

5.1.2. Relationship Between Gender and *Enterobius vermicularis* Infection

The findings of this work appear a high prevalence of the parasites in male more than female, the prevalence was (55.42%) in males and (44.57%) in female, without significant differences between two gender, this findings compatible with findings of Hussein(2015) he was found a rate of infection in males was (59.4%) higher than females (40.6%), as well as agreement with Fan *et al*.(2019) in Taiwan they were scored a high percent of infection (24.50%) in males while (20.31%) in females.
also this findings consistency with study of Ahmed *et al*. (2020) in Nineveh governorate,they were reported a high percent of infection (16.74%) in males while he was found (12.28%) in females, also agreement with Al-Samarai (2020)she was scored 4.38% and 3.37% in male and female ,respectively,

As inconsistency with Al-Daoody and Al-Bazzaz (2020) in Erbil Province where infection rate in females (28.85%) than in males (25.31%)with non-significantly and also disagreement with Al-Shadood(2015) she was scored 53.70% infection rate for females and 46.29%for males with no significant differences between male and female infection in Al-Najaf city from 285 children, also dis agreement with Al-Saqur *et al*. (2016) they were reported a high number of infection (20.171) in females while (16.453) in males in all of Iraq, also disagreement with Musa (2017) she was scored 37,728 males and 40,758 females without significance in all Iraqi governorates, as inconsistency with Al-Ibrahhimi (2019) was 39.8% males and 48.1% females, as dis agreement with Niaz *et al*.(2019) in Pakistan they were recorded 32.18% and37.22% in male and female, respectively.

It is possible that the difference in the percentage of parasitic infections between the sexes can be attributed to the fact that males lead lives that are more likely to be high-stress, active, and full of movement (Khoshnood *et al*., 2015; Cakrlar *et al*., 2019), this may be because males are more likely to engage in activities that put them in contact with unsanitary environments, such as playing or swimming in polluted rivers and eating.

It is possible that gender differences in youngsters play a role in this, with females having more protective personal hygiene behaviours against pinworms than males do. Therefore, they are protected from the pinworm disease (Saki *et al*., 2016; Chen *et al*., 2018; Fan *et al*., 2019).

5.1.3. Relationship Between Residence and *Enterobius vermicularis* Infection.

The incidence of gastrointestinal parasites has been greatly decreased in industrialized nations situated in temperate zones such as North America and Europe owing to high levels of cleanliness, healthcare and education as well as the availability of efficient ant parasites medications (Kubiak *et al*., 2017).

The statically analysis of this study revealed that residence has influences on infection percentage where a urban area are highest (74.21%) than a rural area (25.45%) with significant differences, this finding is near from a study of Fan *et al*.(2019) was (22.95%) in urban area that has more high rate than the rural area (20.69%) while disagreement with results of Wang *et al*.(2016) in china,they were record (4.93%) and (5.41%) in urban and rural areas,respectively.

As inconsistency with Al-Ibrahhimi (2019) was 61.05% in rural and 29.25% in urban, also disagreement with Al-Samarai (2020)she was scored (5.58%) and (2.20)% in rural and urban regions, respectively, as incomputable with Al-Kinany (2020) he was recorded a high percentage of infection (54%) in rural and (46%) in urban regions, also disagreement with Al-Quraishi (2020)she was scored (66.66%) in rural and (33.34%)in urban regions, as disagreement with Al-Rifai *et al*. (2020) was 73.3% in rural and 26.7 % in urban regions.

The highest percentage of infection in urban area may be attributed to it could be congestion with a high numbers of people as well as many of peoples live in random areas of city that currently poor health services in Iraq or may due to mixing sewage with drinking water in this areas (Saki *et al*.,2016).

Also a high numbers of samples that collected from urban area compare with a little numbers of samples from rural area in current study ,on the contrary rural areas have few inhabitants and its open and wide .

5.1.4.Relationship Between Age Groups and Infection with *Enterobius vermicularis*

Because children contribute more to the spread of *E. vermicularis* infection than those of adults, children are seen as the primary population to target, this is one reason why children are the focus of so much attention (Afrakhteh *et al*., 2015).

Rate of *E. vermicularis* in the children community depending on the age groups could be revealed from the results of our epidemiological studies where a findings appears that age groups have an effect on infection percentage ,these findings appears that age group 8-10year has a highest percentage of infection than other age groups while 2-4 year age group has the lowest percentage of infection these findings near a findings of Kadir and Amin (2011) was 29.5% and 21.51% for 6-12 year and 1-6 year age group, respectively, also near from a findings of Al-Shadood (2015) was 37.93% and 13.79% for 6-8 year and 12-14 year age group, respectively, also nearly from with results of Wang *et al*. (2016) they were record lowest infection (2.91%) for 2-4year and (7,50%) for 5-6 year, also nearly from results of with Al-Saqur *et al*. (2016) they were reported a high number of infection(11.004) in 5-14year while lowest number of infection (799) in <1 year, as nearly from findings of Al-Ibrahhimi (2019) who reported a high percent of infection (55.55) and lowest percentage of infection (27.46) in 4-6year and1-3 year, respectively, these findings agreement with a study of Fan *et al*.(2019) they were scored a high percent 32.77% in > 5year and low percent 17.95% in ≤ 5year, also compatible with Al-Samarai (2020) who reported a high percentage of infection (2.48) in 7-8year while lowest percentage of infection (0.29) in <2 year, as well as compatible with a findings of Ahmed *et al*.(2020), they were reported a high percentage (19.32%) in(5-9) age group while incompatible with Hussein (2015), he

was scored a high percent 26.9% in 4year and low percent 1.9% in 12year,as inconsistency with Taylor *et al*.(2018) they were reported a high percentage of infection (38.42) in 3-4year while lowest percentage of infection (6.32) in 7-8 year, as disagreement with Niaz *et al*. (2019) who recorded a little high percentage (39.05) in 1-4year while low percentage (37.63) in 5-8 year, as incomputable with Al-Kinany (2020) who recorded a high percentage of infection (82%) in (12-16) age group and (64.4%) in (8-11) age group, also disagreement with Al-Quraishi (2020) who scored (59.25%) and (3.70%) in (8-18)and (939-45) in age group, respectively, as inconsistency with Labrokasuwng *et al*.(2020) who recorded a high percentage (8.4) in 3-6year while low percentage (4.1) in 7-9 year.

The frequency of pathogenic parasite infections is greatest in children between the ages of 8 and 10, which may indicate that a significant percentage of children in this age groups have a high level of mobility and activity but pay little attention to maintaining cleanliness and hygiene (Emile *et al*., 2013). It is possible that the subsistence nature of parasites, which may cause defects because they can have an impact on patients with lower levels of immunity, is the primary factor contributing to the prevalence of parasite infections in this age group (Khoshnood *et al*.,2015).

The availability of exposed food, which may represent a source of germ infection due to pollution or filthy feeding bottles,

The prevalence of parasites is higher in young people than in other age groups of the population, especially in children (Chen *et al*., 2018).

The lowest percent of infection was found in people aged 2- 4 years, which may be attributed to the fact that this age group moved and conducted business much less frequently.

The differences in the rates between the studies and our results are attributed to the size of the sample, sample type, cultural habits, methodology, occupations, sanitary status, and geographical location (Saki *et al*., 2016)

5.2.Pharmalogical study

5.2.1.The Effect of Cold and Boiling Water Extract Concentrations in Paralysis and Die of *Enterobius vermicularis* Worm in Different Times *In Vitro*

The results of this study demonstrate a high affectivity for a cold water comparative with a boiling water of *citrus limon* plant fruit extract on a paralysis and die of *E. vermicularis* helminths, this finding appears that a cold water of *citrus limon* plant fruit extract was very active and have ability to destroy or killed a worm and this is effect may be that a cold water extract contain active substances that effect on neuromuscular system and led to die this a ability may be because of an extract contain many active components such as phenolic acid, flavonoids, coumarins, vitamins, aminoacids, and carboxylic acids (Klimek-Szczykutowicz *et al*., 2020), or may be because it's contain or rich with nutrients such as vitamin C (ascorbic acid), carbohydrates and minerals (Huang *et al*., 2019) or may be because it is rich with phenolic (acids ferulic acid, synapic acid) that alters a PH media of worm and leads to kills it,or a phenolic compound adhere with tegument cells of helminths and stopped it is activity , where a phenolic compounds are dissolve in water (Anku *et al*., 2017), or an activity of *C. limon* plant fruit a cold water extract may be due to that active components or substances high decomposes in cold water that lead to concentration of this substance that effect on parasite more than in boiling water because a hot may be broke or damage an effective substances in plant that lead to reduces its activity on worms.

The statically analysis appears that a high effect on a paralysis and die of *E. vermicularis* helminths which was by a highest concentration (300mg/ml) for cold *C.limon* extract with significance differences, this result shows the great effectiveness for *C. limon* plant extract fruits with a high concentration ,this results fits with a finding of Sravanthi *et al*. (2020) they were get on a great anthelmintic activity of aqueous extract for *C. limon* plant on adult earthworm *E. Foetida* in (20mg/ml) concentrations, this effect of *C. limon* plant fruits extract may be attributed to it is a high amounts of components of phenolic compound as phenolic

acid, hydroquinone, resorcinol, thymol, phenolic acids, tannins, stilbenes and lignans (Zandalinas *et al*., 2017; Anku *et al*., 2017; Upadhyaya *et al*., 2019).

The high phenolic component content of *C.limon* is largely responsible for the beneficial biological activity that it has (Kuo *et al*., 2017;Makni *et al*., 2018), flavonoids such as diosmin, hesperidin, and limocitrin as well as phenolic acids such as ferulic, synapic, p-hydroxybenzoic acids and other biologically active compounds that are rare in other compounds have been reported to have multiple biological effects when they are present in phenolic compounds, these effects have been attributed to phenolic compounds (Elija *et al*., 2017; Kuo *et al*., 2017; Makni *et al*., 2018), the pharmacological potential of *C. limon* is determined by its rich chemical composition, which is the reason for the importance of C. limon in the food and cosmetics industries (Otang and Afolayan, 2016; Riaz *et al*., 2014).

The wider pharmacological actions of *C.limon* extract used as hepatoregenerating , antifungal , antibacterial, antitumor, anti-inflammatory, and cardioprotective activities (Russo *et al*., 2015), because of their nutritional value, their anthelmintic activity, and their antioxidant capacities, *Citrus limon* fruits have been shown to have a significant positive influence on the public health (Elija *et al*., 2017, Munne *et al*., 2011).

The extract of Date Vinegar have effective roles in a paralysis and die of *E. vermicularis* helminths with increase of concentration and period time, where was the water cold extract in 300 mg/ml concentration has greater impact than hot water extract , this result is fits with result of Sadjjadi *et al*. (2006) they were get on a high effective of cold acetic acid on giardiacidal activities that was 40.6% after 3 hours was at 24˚C, also agree with finding of Beyhan *et al*. (2016) they were founds that cold vinegar have an effective roles in kills all an eggs of *A. lumbricoides* at 5% concentration after 30 minutes, this activity of cold Vinegar may be due to a presence of phenolic substance promotes antioxidant action (Luzón-Quintana *et al*., 2021), or may be attributed to that Vinegars contain substances which have

synergistic effects to change the body as sinapic acid reacted with ferulic acid, protocatechuic acid reacted with dihydro ferulic acid, salicylic acid with protocatechuic acid showed antagonistic effect (Xia *et al*., 2020) or may be due to a presence of organic acids and melanoidins ,tetramethylpyrazine and lovastatin have antioxidant effect, results to reduction risk of diseases (Hemke *et al*., 2019 ; Xia *et al*., 2020) or may be due to a presence organic acids, phenolic acid, and amino acids proposed which the amino acids have a supported effect on the phenolic compounds, however, the organic acids demonstrated an antagonistic activity (Zhang *et al*., 2019).

Therapeutic effects of vinegar arising from consuming bioactive components including energy production, metabolism regulation, immunity modulation, anticoagulation, and antioxidation (Budak *et al*., 2014; Gil *et al*., 2020; Xia *et al*., 2020) differential vinegar varieties having different impact on human health as well as body metabolism (Luzón-Quintana *et al*., 2021) vinegar had greater activity than other simple disinfectant material (Sadjjadi *et al*., 2006), vinegar act as medicinal and therapeutic potential advantages as anti-carcinogenic, anti-glycemic, anti-cardiovascular, antioxidant, antimicrobial (Chen *et al*., 2016; Hemke *et al*., 2019).
From this study appears that a best water extract that lead to a paralysis and die of *E. vermicularis* helminths in short period time was *C. limon* plant extract that shows an efficiency of active substances found in *C. limon* plant more than active substances found in vinegar extract.

5.2.2.The Effect of Plant Type Factor on Paralysis and Die of *Enterobius vermicularis* Helminths *In Vitro*

From a results of this appearance that *C. limon* plant is a best used plant in a paralysis and die of *E. vermicularis* helminths, this findings confirms an effectiveness of *C. limon* plant, this finding is fits with a finding of Khatiwora (2018), Upadhyaya (2018), Sravanthi *et al*. (2020) Shija *et al*. (2020),they were get on a high effective of *C. limon* plant that have an effective roles against parasites, this finding may be due to *C. limon* plant contains a high amounts of limonene

compound that possess antimicrobial activity, (Ibanez *et al*.,2020), or may be attributed to vitamin C Which strengthens the immune system, acts as an antioxidant and protects cells from radical damage (Khatiwora,2018), or to that *C. limon* extracts contain many effective minerals that may be synergic with other compound found in *C. limon* extracts as phenols , terpiens and flavenoids that effect on parasites, *C. limon* extracts are found to do superior potential anthelmintic or antiparasitic activity (Khatiwora , 2018; Shija *et al*., 2020).

5.2.3.Effect of Water Extract Concentration Type Factor on a Paralysis and Die of *Enterobius vermicularis* Helminths *In Vitro.*

The statics analysis demonstrates that extract concentration factor important where was a high concentration (300mg/ml) for water extract has great and effective roles on a paralysis and die of *E. vermicularis* helminths comparative with others concentrations figure (4-3) , this findings is fits with a results of Sadjjadi *et al*. (2006), Beyhan *et al*.(2016) Khatiwora (2018), Upadhyaya (2018), Sravanthi *et al*. (2020) and Shija *et al*. (2020) they proved and confirms that a great concentrations possess a high effects on parasites ,this effects may be attributed to that *C. limon* extracts contains valuable source of phenolic and other biologically active compounds (Upadhyaya, 2018) where a high concentration makes an amount of active substance is more and therefore it is effect is greater.

5.2.4.The Interference Effects of Albendazole Drug in Gram /ml Concentrations on Paralysis and Die for Worm *In Vitro*

The static analysis for findings of interference effects of albendazole drug concentrations demonstrated that albendazole drug possess a high activity on a paralysis and die of *E. vermicularis* helminths especially with 300 mg\ml concentration where its led to a paralysis and die in very short period of time, this result are fits with Otu-Bassey *et al*. (2011), Al- Madhi (2016), and Temsah *et al*. (2021), these researchers founding that a high activity of albendazole drug against various helminths ,these effective of albendazole drug may be attributed to a its effect on preventing the molecules absorption which leading preventing of the

parasite growth (Verrest and Dorlo ,2017) , or by action of mechanism of the binding inside the cell microtubules and doesn't allowing to the elongation (Verrest and Dorlo ,2017; Hong,2018).

Albendazole characterized by higher dissolution properties in fats medium (Sawatdee *et al*.,2019), and very dynamic distribution and high rate of metabolism of albendazole drug (Cowan *et al*., 2017),albendazole belongs to the benzimidazole class of drugs and is used to treat a wide range of parasitic diseases (Xing *et al*.,2018),it is generally effective and safe drugs (Hong, 2018), also it is broad spectrum anthelmintic effect in helimenthiasis treatment, therefore, it used for treatment of the toxocariasis, echinococcosis, and gnathostomiasis, cysticercosis, and taeniasis (Albonico *et al*., 2016).

5.2.5.The Interference Effect of Secondary Compounds Extracts Concentrations for *Citrus limon* Plants *In Vitro*

The present study proven that terpenoids compound of *C. limon* plants are more influential than other secondary compounds it led to worm paralysis and death in a short period of time, this finding appears that terpenoid compounds may be effect by it's a high amount of limonene that have ability to dissolve a cholesterol of cells (Sun, 2007), that led to die of cell or may be that terpenoid interesting activity could be related to the lipophilic properties of it that were shown to contribute in the disruption of parasite intracellular metabolic pathways (Maaroufi *et al*., 2021).

This effect of terpenoids compounds may be due to a presence of pipecolic acid, pulegone, scopoletin, carvone, terpinene, myrcene, isopiperitenol, linalool, menthone in *C. limon* plant which observed their effects (Gupta *et al*., 2017), or to efficacy of α-pinene, γ-terpinene, β-pinene, limonene linalool and menthone which are detected in the lemon (Gupta *et al*., 2017; Prakash, 2018),

The effects of terpenoids Secondary compounds may be attributed to interactions among components are assumed to be mediated by the emission of terpenoids (Boncan *et al*., 2020), terpenoids have been widely considered as therapeutic agent (Prakash, 2018), as well as recent studies showed that terpenoids could also

individually or in mixture contribute to the whole antioxidant ability, also terpenoids as important bioactive constituents of essential oil (Stephane and Jules, 2020), from these results we conclude that best secondary compounds that lead to a paralysis and die of *E. vermicularis* helminths in short period time was terpenoids that found in *C.limon* plant.

This study appears that phenolic compounds has clear activity on paralysis and die of *E. vermicularis* helminths where was a time for a worm to paralysis was 32.33 minute and for worm to die was 53.00 minute, this effect of phenolic compounds probably attributed to its rich with tannins (Anku *et al*., 2017), tannin affects protein composition in worms exposed to high concentrations and thus affecting the neural receptors which leads to the worm's paralysis and death (Al-Madhi, 2016), tannins polyphenols binding to protein through hydrogen bonds and forming a tannin–protein complex (Avila *et al*., 2020), or tannins destroys an organism's cell membrane through its effect on the fats and proteins in it and then the organism loses its ability to grow or penetrates a cell membranes and obscures the active sites of some enzymes inside the cell which is necessary for growth (Mahmoud and Ali, 2012; Inabo and fathuddin, 2011), phenolic compounds are recognized as being responsible for an antioxidant and antihelminthic ability (Anku *et al*., 2017; Stephane and Jules, 2020).

The results of this study appear that alkaloids compounds have antihelminthic activity on paralysis and die of *E. vermicularis* helminths, this activity of alkaloids compounds can be attributed to that alkaloids may be disrupting the action of the enzyme, receptors and proteins by forming hydrogen bonds with this compound where they have proton receive N atom, and amine H atoms (Cushnie *et al*., 2014), such as pergularinine and tylophorinidine alkaloids both of them decrease the dihydrofolate reductase activity that are responsible for nucleic acid synthesis (Rao and Venkatachalam, 2000),or an alkaloids may be binds to protein that important in cell division and leading into enzymes and proteins inhibition (Boberek *et al*., 2010), or an alkaloid may be acts through a detergent-like mechanism that led to

disruption of outer membrane of microorganisms (Alhanout *et al*., 2010), also an activity of alkaloids presence were caused due to virulence factors inhibition in the parasite (Yang *et al*., 2011).

Alkaloids have several effects such as central nervous system stimulants, inflammatory, vasoconstrictor, anticholinergic and antimalarial (Cushnie *et al*., 2014; Almeida *et al*., 2017), the anti-inflammatory activity of alkaloids, involving inhibition or regulation of important inflammation mediators (Almeida *et al*., 2017).

This is results proven that terpenoid compounds it is the most influential of other secondary compounds and have a significant clear impact on a paralysis and die of *E. vermicularis* helminths therefore terpenoid compounds were chosen as the best secondary compounds, including the essential oil for GC-MS analysis (Gas Chromatography Mass Spectroscopy) and extract the most effective substance in essential oil to try it on *E. vermicularis* helminths *in vitro*.

5.2.6. Concentration Type Factor Effects for Secondary Compound for *Citrus limon* Plants on Paralysis and Die for Worm *In Vitro*

Results or findings of this study appears that a well concentration of secondary compound extracts was a high concentration (300mg/ml) that possess an effective roles on a paralysis and die of *E. vermicularis* helminths comparative with others concentrations, this results was agree with findings of Aziz *et al*. (2014), Yang *et al*. (2014), Al- Madhi (2016), and Lalthanpuii and Lalchhandama (2020) they were proved that concentration of secondary compound extracts have clear and a great effect on a paralysis and die of *E. vermicularis* worms, this finding may be attributed to that a high concentration offers a great adhesive for active components in secondary compound with a cells of parasites and kills it or a high concentration limonene in the fruits (Soulimani *et al*., 2019).

5.2.7.Isolation and Diagnosis of Limonene Compound from Terpenoid Compounds Extracts of *Citrus limon* Plants by GC-MS and Show its Impact on Paralysis and Die of *Enterobius vermicularis* Helminths *In Vitro*

The results of gas chromatography mass spectroscopy GC-MS technical showed that terpenoid compounds of Iraqi *C. limon* plants fruit contain limonene compound where it was a retention time of this compound very close to a retention time of stander limonene compound .

This findings was similar to findings of Wu *et al*. (2014) in Taiwan, it used mass spectrometry detector and gas chromatography in detection of component *C. limon* plant EOs, and that means that limonene was present in *C. limon* plant EOs that accounting 57.71 area, also this findings was similar to findings of Shakir and Salih (2015) in Iraq ,they are analysis essential oil of *Citrus* sp. plant fruits peel (orange, lemon, mandarin) and its contents of limonene is (83.2189, 65.2867and 83.0271) respectively, they showed that limonene compounds is the most abundant component in essential oil of *C. limon* plant fruits peel where that analysis by GC-MS technical is mainly composed of limonene, linalool, citronellal, nerol, geranial as major compound ,among many other components, also this findings was fits to findings of AL-Jabri and Hossain (2016) they were analyzed essential oils by GC-MS technical where they found a main bioactive compounds with high content in Omani sweet lemon essential oil was limonene compound (84.73%) and Omani sour lemon essential oil was composed of limonene (53.57%), as well as Paw *et al*. (2020) they were extracted peel essential oil of *C. limon* and analyzed by GC/MS technical to identified a compounds that was limonene (55.40 %) and neral (10.39 %) were found as major compound followed by *trans*-verbenol (6.43 %) and decanal (3.25 %), also, a study was done on *C.limon* grown in Iraq by Kaskooz (2019) to appear the composition and properties of the EOs that was extracted by the hydro-distillation method and analyzed by GC-MS, the results demonstrated that the major constituents of the essential oil of *C.limon* peels were limonene (29.52%), -pinene (23.89%), citronellal (11.53%) and thymol (9.79 %).

Gas Chromatography Mass Spectroscopy, quick and reliable platform for EOs analysis in both qualification and quantification (Wu *et al*., 2014), it is a hyphenated system is a very compatible technique and the most commonly used technique for

the identification and quantification purpose (Yamuna *et al*., 2017), it is a techniques which have high sensitivity for natural essential oils due to its composed of many ingredients (Wu *et al*., 2014).

Many GC columns segregate compounds based on their boiling point since GC-MS is an important technique in current analytical chemistry (Yamuna *et al*., 2017; Jones, 2019), this is because low-boiling substances move quicker and have less reactivity than high-boiling substances, the discovery of warfare agents and explosives, the examination of athletes urine for illegal performance-enhancing drugs, the screening of urine samples from Mars, and the creation of novel medications are all examples of uses for this technology, simply diluting the natural EOs with methanol led to the production of the finished product, but platform's efficiency was increased because to a GC separation gradient that was just a few steps long, which was coupled with an autosampler (Wu *et al*., 2014).

In this work, its demonstrated that GC-MS is an extremely accurate and technically advanced method for the isolation of components of secondary compounds, particularly the limonene compound, which is the most prevalent component of Iraqi *C. limon* plant fruit.

The limonene compound led to paralysis and die of *E. vermicularis* helminths especially with 100,200,400 µ/ml concentration where its led to a paralysis and die of worm with high efficient indicates that limonene compound has therapeutic capacity against this parasite , an activity of limonene compound may be attributed to that limonene effect on tegument then muscular and nervous system of worm by dissolve a cholesterol of cells and led to paralysis where its limonene that an excellent solvent for cholesterol, limonene has been used clinically to dissolve cholesterol-containing gallstones (Sun, 2007), or to that limonene raised an acidity that effect on metabolism enzyme and receptors of worm cells.

Limonene was the primary chemical component of the essential oil extracted from *C. limon* peels, this oil, which has a wide range of therapeutic applications and

is thus valuable to the pharmaceutical industry, may be used in the treatment of a variety of conditions (Paw *et al*., 2020).

This monoterpenes wide range of possible mechanisms of action, along with the fact that it is both safe and abundant in nature, make it a promising candidate for use as a preventive agent, additionally, it might be useful as a complementary strategy to conventional therapeutic drug treatments that focus on reducing inflammation and treating infections (Soulimani *et al*., 2019),according to Ibánez *et al*. (2020) research , the most important new uses for limonene in the food business are as an antibacterial, herbicidal, and antioxidant agent.

The broad use of limonene in soft drinks, cosmetics and many other flavoring products has raised interest in the antimicrobial, anticancer, toxicity, antiparasitic and many other properties of limonene (Erasto and Viljoen, 2008; Ibáñez *et al*., 2020), *C. limon* peel essential oil limonene could also be implemented for the herbal formulation of cost effective, easily available antimicrobial and antioxidant drugs (Paw *et al*., 2020), it is generally recognized as safe for human consumption as a synthetic flavoring substance (Soulimani *et al*., 2019; Ibáñez *et al*., 2020).

The maximum safe daily intake (TDI) of limonene was calculated to be 0.27 milligram per kilogram of the animal's body weight. The LD50s of oral d-limonene in mice and rats often surpass 5 gram per kilogram of the animal's body weight, the rabbit has a dermal LD50 that is more than or equal to 5 gram per kilogram of body weight, the median lethal dose (LD50) after intraperitoneal administration is 1.3 gram per kilogram in mice and 4 gram per kilogram in rats, rats have an intravenous LD50 of 0.1 gram per kilogram of body weight (Soulimani *et al*., 2019), in this particular research project, we found that the limonene molecule had a highly efficient action against *E. vermicularis* adult helminths when tested in vitro.

5.2.8.Effect of Albendazole with Microgram Unit on Paralysis and Die of *Enterobius vermicularis* Helminths *In Vitro*.

Statically, a results of the albendazole drug's activity analysis showed a great efficiency on a paralysis and die of *E. vermicularis* helminths with 400and 200µ /ml *in vitro,* this result are fits with Al- Madhi (2016)and Temsah *et al*. (2021)who confirmed that the drug albendazole has a significant activity against a variety of helminths.

This activity of the albendazole drug may be due to the fact that albendazole results in degenerative alterations in the tegument and intestinal cells of the worm by reducing the worm's ability to produce energy, which ultimately results in the immobilization and death of the parasite, it does its job by binding to a colchicine-sensitive site on tubulin, which prevents the protein from being assembled into microtubules, since cytoplasmic microtubules are essential for promoting glucose uptake in the larval and adult stages of susceptible parasites, this results in the parasites glycogen stores being depleted, because to degenerative alterations in the endoplasmic reticulum and the mitochondria of the germinal layer, as well as the subsequent release of lysosomes, the generation of adenosine triphosphate, which is the energy the helminth needs to survive, is reduced (Houpt and Chaudhry, 2009; Hong, 2018; Sawatdee *et al*., 2019).

Albendazole drug has a broad-spectrum anthelmintic agent with good efficacy in the treatment of echinococcosis, hydatid cysts, and neurocysticercosis caused by nematodes and cestodes (Hong, 2018; Sawatdee *et al*., 2019).

Eating meals with high in fat dramatically increases absorption, which is critical for tissue parasites, absorption is rapid in both people and animals; maximal blood levels of drug are reached within two to three hours (Ryan, 2018; Hong, 2018), when it reaches the plasma, it is rapidly metabolized in the liver to albendazole sulfoxide, and then it must finally undergo biotransformation by P-450 enzymes in order to become the inactive metabolite albendazole sulfone ,but a portion of albendazole is metabolized in the intestinal mucosa during the absorption process, and this occurs before it reaches the liver (Hong, 2018; Sawatdee *et al*., 2019; McCarthy and Moore, 2020).

5.3. Molecular Study

5.3.1.Molecular Characterization of *Enterobius vermicularis*

To better understand the evolutionary connections of nematodes, great research have used a variety of data types, including DNA sequences (Kern *et al*., 2020), as molecular techniques have been developed to enhance diagnosis of pathogens, validating of PCR technique when it is using for diagnosis is challenging ,the using of computer modeling for primer designing to enhance PCR conditions, sensitivity, and specificity, the diagnostic purposes require using of primers that target housekeeping genes or ribosomal ribonucleic acid that common to numerous species to identifythe pathogens at genus level (Leblanc *et al*., 2014).

Analysis of DNA sequences is of importance in confirmation of classical morphology- based identifications of nematode species and in reconstruction of their phylogenetic relationships with available sequences in GenBank (Zeng *et al*., 2015), in the case of the phylum Nematoda, inferring precise phylogenies may reveal substantial insights into the development of this amazing group as well as the variety of its members, one of the most frequent, environmentally diversified, and diverse in terms of species, nematodes is one of the animal groups that may be found all over the globe (Kern *et al*., 2020).

5.3.2. Diagnosis of *Enterobius vermicularis* by PCR Technique.

There have been very few molecular investigations conducted to characterize the genotype of *E. vermicularis* (Tavan *et al*., 2021), in the domains of population genetics and systematics, the use of molecular and analytical technologies enables a reconstruction of evolutionary links between parasites over a broad variety of time and geographical scales, which improves our capacity to detect parasites, when attempting to define the genetic variety of human pinworms, molecular features of *E. vermicularis* isolates serve as an excellent place of departure (Kubiak *et al*., 2017).

Molecular study findings that conducted by using polymerase chain reaction technique for amplification cox1 mitochondrial genes with 407 bp of *E. vermicularis* revealed that samples of adult worm and ova is positive with genetic variations was (0.0080-0.0020), these verified a presence of *E. vermicularis* worm in a sample , these results agreement with findings of Piperaki *et al*. (2011) their results appear a bands of cox 1 genes by 333 nucleotide long fragments with genetic variations , also with results of Ferrero *et al*. (2013) their results clarify a bands of cox 1genes by 333 nucleotide long fragments, as with a study by Foitová *et al*. (2014) in Indonesia on *cox*1 gene with genetic variations was (0.036) , as well as with findings of Hagh *et al*. (2014), they were revealed that the all samples belonged to haplogroup B depend on Cox1 genes ,also with results of Kubiak *et al*. (2017) their findings appears existence of a three different haplotypes of pinworm by using cox1 genes with genetic variations (0.01) , as with Tomanakan *et al*. (2018) they were get on a bands of cox1 gene with genetic variations (0.9888) , as well as with findings of AL-Ibrahimi (2019) her results appear bands of cox1 gene of *E.vermicularis* parasite based on the 301pb, also with results of Al-Samarai (2020) her findings appears bands of cox1 gene of based on the 566 pb, with genetic variations was (0.0020), also with Tavan *et al*. (2021), they were amplified 390 bp piece from cox1 gene with genetic variations was (0.02),

The cox1 gene used for detected many of living organism because a cox1 gene has been reported to display high variability than the other regions and proposed to phylogenetic studies (Hagh *et al*., 2014),also can be coded on one strand (Kim *et al*., 2018), as well as a valuable tool for worms and has been frequently employed to resolve uncertainties within this group of organisms, mitochondrial genes (kern *et al*., 2020), some researchers claim that the biological development of a cox1 gene is fast enough to differentiate between related species and examine intraspecific variation (Lin *et al*., 2015).

The cox1 region is very useful for distinguishing between different species of vertebrates and invertebrates (Oba *et al*., 2015; Trivedi *et al*., 2016),the DNA barcoding technique that makes use of cox1 is one that looks to have a greater evolutionary indication than the other mitochondrial genes (Rodrigues *et al*., 2017).

5.3.3. Phylogenetic Analysis.

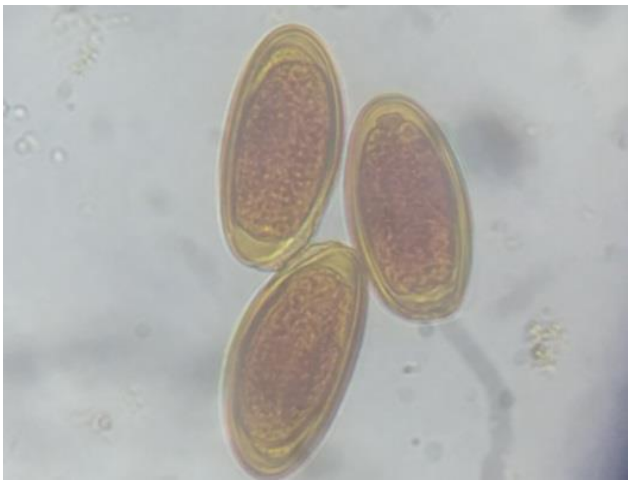
Researching and analyzing the mtDNA genes of pinworms has shown to be an effective method for determining identities, researching evolutionary history, determining regional variances within the same species, and determining population differences (Kubiak *et al*., 2017;Fererro *et al*., 2013), additional research on Oxyurids from a variety of hosts and geographic locations helps improve our knowledge of the links between these parasites as well as their evolutionary history (Sorour *et al*., 2020), the nematode has many phylogenetic markers. mitogenomes of the nematode produced alternative tree as compared with nuclear gene, nematode mitogenomes have been used to infer the evolutionary relationships between nematode species (Kern *et al*., 2020).

In present molecular study tests and from a cox1 genes sequences analysis by PCR technique that confirmed a diagnosis of *E. vermicularis* , a local *E. vermicularis* isolates (IQH-No.1-IQH-No.6) were show closed related to NCBI-Blast *E. vermicularis* of Iran isolate (MH802611.1) at total genetic changes or variations (0.0020-0.0080) , these findings were these results were agree with results of Ferrero *et al*.(2013)they were shows pinworms in Denmark closely related to samples collected in Germany, Greece and Japan cox1 mitochondrial gene,also agree with results of Kubiak *et al*. (2017) their results appears a sequences homogeneous with *E. vermicularis* isolates of the human from Japan, Germany, Denmark, and Greece with genetic variation 0.01, as well as with findings of Tomanakan *et al*. (2018), who found a genetic variation in phylogenetic analysis and similarity in Thailand isolates with isolates from Japan and Korea, Iran, Czech Greece, Denmark and Sudan , as well as with findings of Tavan *et al*. (2021), their results showed 0.02% a genetic variation and

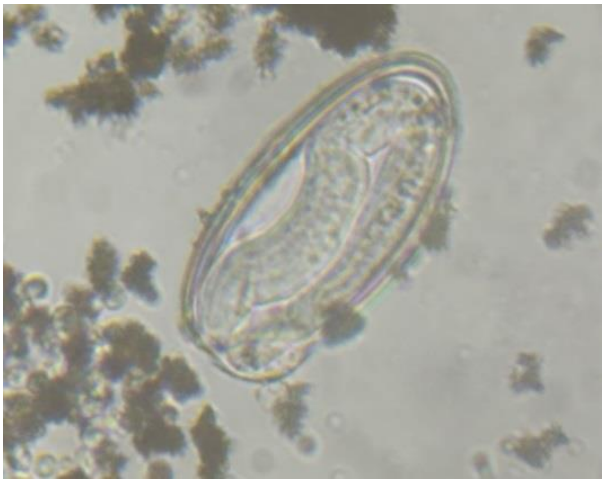
homology with isolates from Greece . also with results of AL-Ibrahimi (2019) who found genetic homology of her study isolate with isolate from Iran and Hunan and she was found a genetic variation, as well as Al-Samarai (2020), her results were showed a genetic related to NCBI-BLAST *E. vermicularis* at total genetic changes (0.0020%) and a local isolates were showed 99%homology identity to the NCBI-BLAST *E. vermicularis* isolates.

The use of whole sequences of mitochondrial genomes is one approach that has been extensively utilized to many different nematode branches where the connections between the species were unknown (kern *et al*., 2020), within Oxyurids, the cox1 gene is the most conserved of all genes (Zhang *et al*., 2015), mt DNA genes were shown to be effective genetic targets for the identification and research of medically significant pinworms (Sorour *et al*., 2020), amplification of some partial mtDNA genes confirms a species and helps to make conclusions about its links with other oxyurids (Sorour *et al*., 2020; Kern *et al*., 2020), the mitogenome has the potential to offer resolution for an extremely extensive variety of phylogenetic trees, ranging from divergence ages that are quite recent within populations of a single species to divergence times that are extremely ancient within an entire phylum (Kern *et al*., 2020).

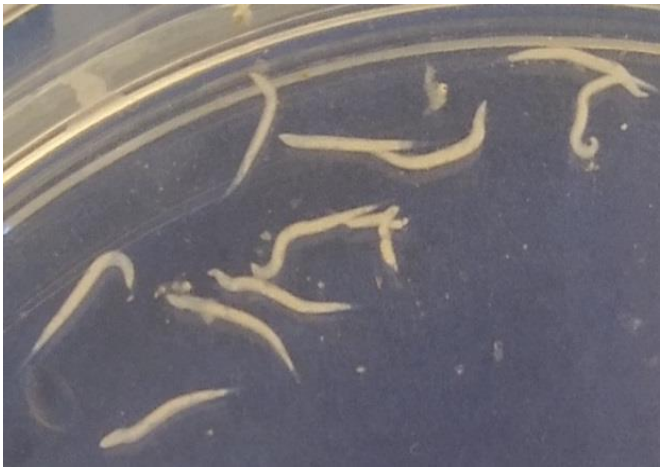
Appendix (1):*E.vermicularis* ova at (400X)



Appendix (2):*E.vermicularis* larvae inside ova(400X)

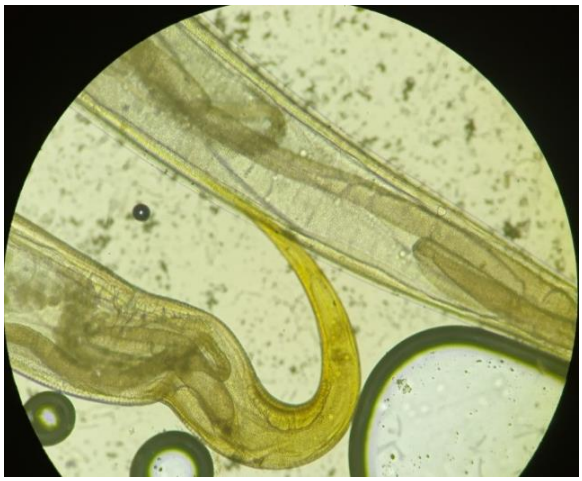


Appendix (3)Adult females of *E.vermicularis* in petridish .



Appendix (4) Adult female of *E.vermicularis*.

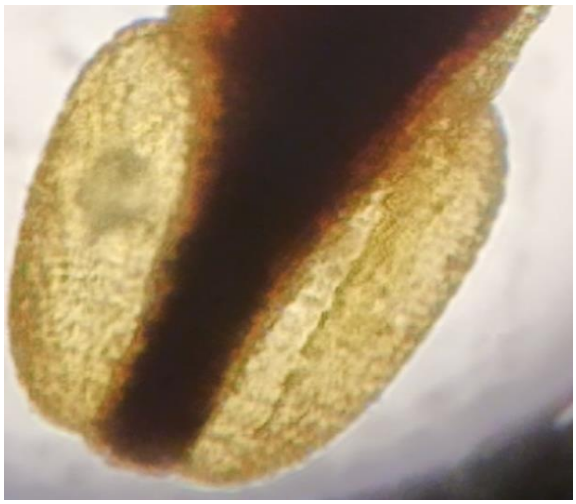
Appendix (5):Female *E. vermicularis* end portion of body (200X)



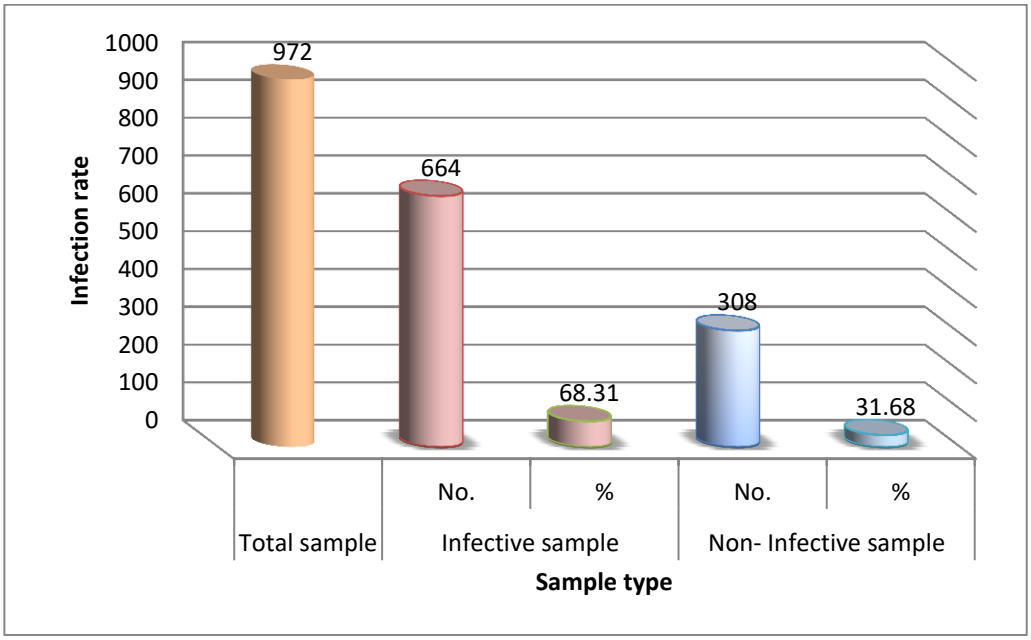
Appendix (6)Mid portion of *E.vermicularis* body(200X)

Appendix (7):Adult female of *E. vermicularis* (40X).

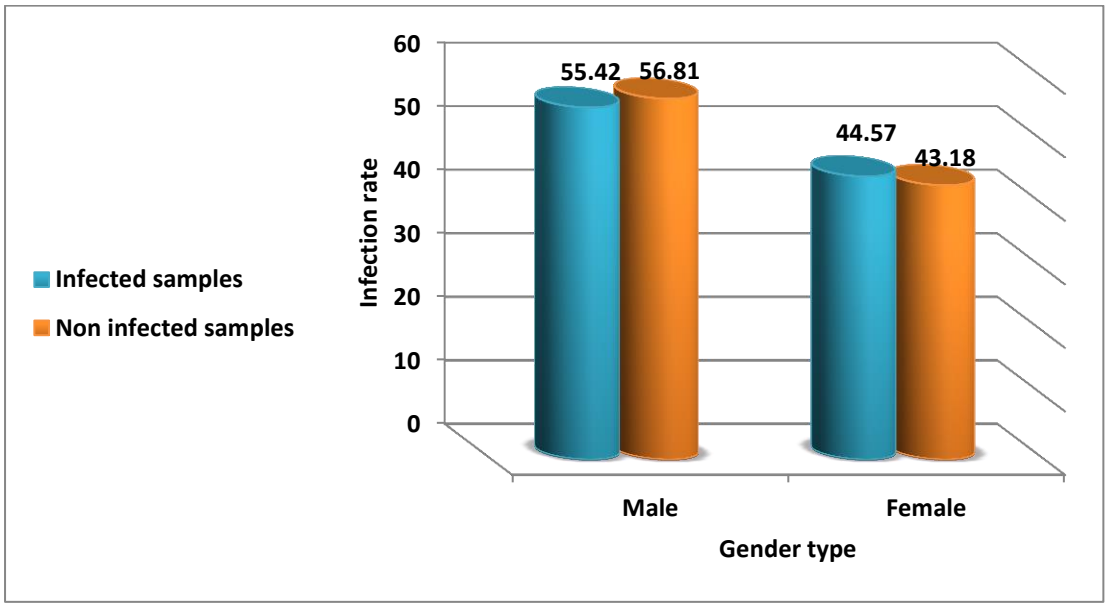
Appendix (8)Head of *E.vermicularis* female (200X)



Appendix (9) Percentage of infected children with *E. vermicularis* using a lugol's iodine stain and scotch tape method.

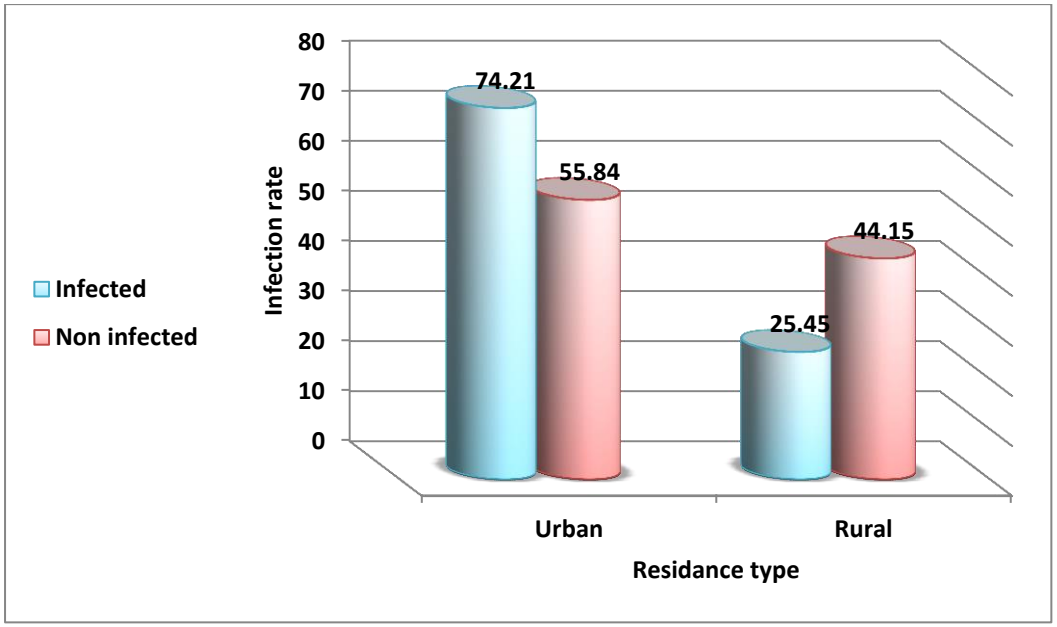


Appendix (10) Numbers and percentages of patients infected with *E. vermicularis* according to the gender.



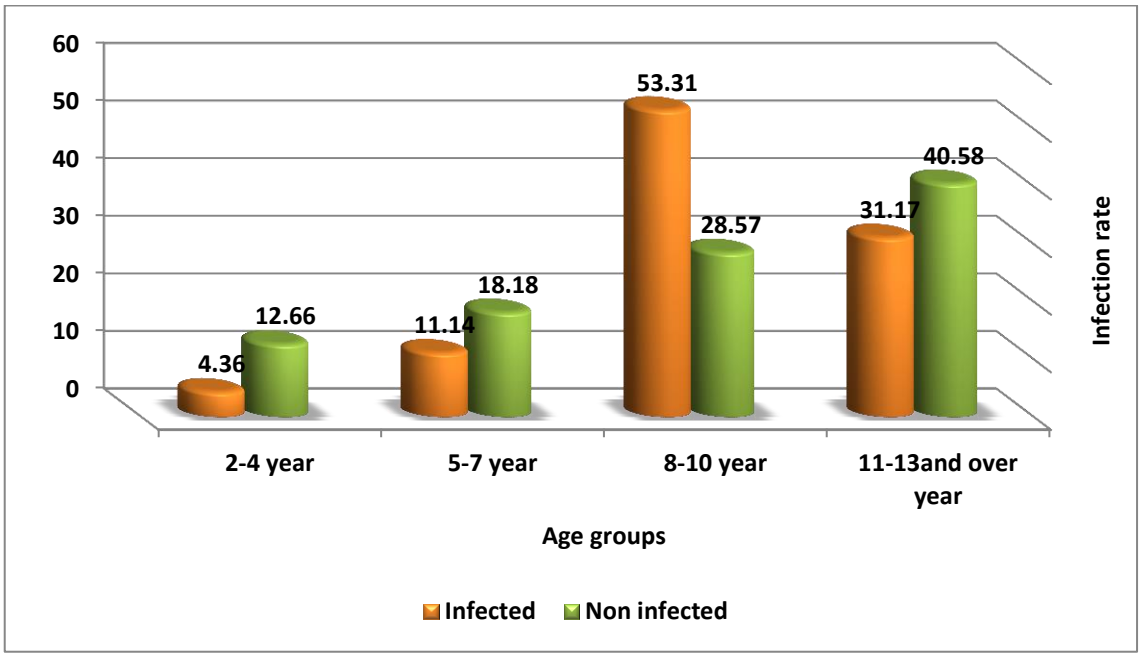
P.value = 0.683

Appendix (11)Distribution of *E. vermicularis* infection among children according to the area residence.



P.value = 0.0001

Appendix (12)Relationship between the age groups and the infection with *E. vermicularis* among children.



P.value =0.0001

Appendix (13)The effect of plant type factor of water extract of *Citrus limon* plant fruits and Veingar date on worm paralysis and die *In vitro*.

Plant type	Time per minute for worm to paralysis Mean	±S.D.	Time per minute for worm to die Mean	±S.D.
Limon plant	405.87	120.2	477.00	129.9
Veingar date	504.58	111.6	609.58	112.3
LSD at probality level 0.05	10.23		12.60	

Appendix (14)The effect of extract type factor of aqueous extracts of *C. limon* plant fruits and date veingar on worm to paralysis and die *In vitro*.

Extract type	Time per minute for worm to paralysis Mean	±S.D.	Time per minute for worm to die Mean	±S.D.
Cold extract	441.88	118.4	514.33	125.7
Hot extract	468.58	114.4	572.25	118.3
LSD at probality level 0.05	10.23		12.60	

Appendix (15)The effect of concentration type factor for aqueous extracts of *C. limon* plant fruits and date veingar on worm to die and paralysis *In vitro*.

concentration mg/ml	Time per minute for worm to paralysis Mean	±S.D.	Time per minute for worm to die Mean	±S.D.
300	86.33	12.6	150.08	22.3
200	121.17	15.9	197.50	27.4
100	200.83	34.3	283.08	41.8
control	1412.58	9.1	1542.50	10.8
LSD at probality level 0.05	14.47		17.82	

Appendix (16)The effect of Albendazole drug concentrations on worm paralysis and death *In vitro*.

Concentration mg/ml	Time per minute for worm to paralysis	±S.D.	Time per minute for worm to die	±S.D.
	Mean		**Mean**	
300	48.00	.6	70.00	1.0
200	61.00	.6	116.00	1.0
100	80.00	.6	134.00	13.0
control	1424.33	21.2	1544.00	33.9
LSD at probality level 0.05	34.55		34.25	

Appendix (17)Secondary compound type effect for *C. limon* plants on paralysis and die for worm.

Secondary compound type	Time per minute for worm to paralysis Mean	±S.D.	Time per minute for worm to die Mean	±S.D.
Terpenoids compound	**367.33**	177.4	**426.17**	197.4
Phenolics compound	**391.33**	179.9	**443.67**	193.0
Alkaloids compound	**396.00**	176.1	**454.17**	189.2
LSD at probality level 0.05	**12.45**		**13.47**	

Appendix (18)Concentration type effect factor for Secondary compound for *C. limon* plants fruits on paralysis and die for worm.

concentration mg/ml	Time per minute for worm to paralysis Mean	±S.D.	Time per minute for worm to die Mean	±S.D.
300	30.11	3.2	49.67	4.4
200	42.56	5.2	68.56	7.0
100	61.00	5.8	96.56	8.1
control	1405.89	10.1	1550.56	9.9
LSD at probality level 0.05	14.38		15.55	

Appendix (19)Cox1 gene sequences alignment of *Enterobius vermicularis* worm.

The analysis of mitochondrial Cox1 gene sequences for six local *E.vermicularis* isolates and compared with NCBI-BLAST *E. vermicularis* related Genotypes isolates was carryout as found in the following figers:

***E. vermicularis* isolate IQ-No.1 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial**

Sequence ID: MZ505531.1 , 1Length: 298 bp DNA linear .

Score	Expect	Identities	Gaps	Strand
534 bits(289)	5e-156	295/298(99%)	0/298(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGT
TAGTCAT 60

Sbjct 1 .....**T**..... 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATG
ATTTATGCT 120

Sbjct 61 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTA
CTATTGGT 180

Sbjct 121 **T**.....**G**..... 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTG
TGCCAACT 240

Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTT
GTGCAGC 298

Sbjct 241 298

The figure appears alignment a sequences analysis for Cox1 gene in *E. vermicularis* local isolates number(1) that recorded in NCBI-BLAST.

***E. vermicularis* isolate IQ-No.2 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial**

Sequence ID: MZ505532.1, Length: 294 bp DNA linear.

Score	Expect	Identities	Gaps	Strand
532 bits(288)	2e-155	292/294(99%)	0/294(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGT
TAGTCAT 60

Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATG
ATTTATGCT 120

Sbjct 61 .....**A** 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTA
CTATTGGT 180

Sbjct 121 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTG
TGCCAACT 240

Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTT
GTG 294

Sbjct 241 .....**T**..... 294

The figure appears alignment a sequences analysis for Cox1 gene in *E. vermicularis* local isolates number(2) that recorded in NCBI-BLAST.

***E. vermicularis* isolate IQ-No.3 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial**

Sequence ID: MZ505533.1 , Length: 295 bp bp DNA linear.

Score	Expect	Identities	Gaps	Strand
534 bits(289)	5e-156	293/295(99%)	0/295(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGT
TAGTCAT 60

Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATG
ATTTATGCT 120

Sbjct 61 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTA
CTATTGGT 180

Sbjct 121 .....**C**..... 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTG
TGCCAACT 240

Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTT
GTGC 295

Sbjct 241 .....**T**..... 295

The figure appears alignment a sequences analysis for Cox1 gene in *E. vermicularis* local isolates number(3) that recorded in NCBI-BLAST.

***E. vermicularis* isolate IQ-No.4 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial**

Sequence ID:MZ505534.1 , Length: 296 bp bp DNA linear.

Score	Expect	Identities	Gaps	Strand
542 bits(293)	3e-158	295/296(99%)	0/296(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGT
TAGTCAT 60

Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATG
ATTTATGCT 120

Sbjct 61 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTA
CTATTGGT 180

Sbjct 121 .....**C**..... 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTG
TGCCAACT 240

Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTT
GTGCA 296

Sbjct 241 296

The figure appears alignment a sequences analysis for Cox1 gene in *E. vermicularis* local isolates number(4) that recorded in NCBI-BLAST.

***E. vermicularis* isolate IQ-No.5 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial**

Sequence ID: MZ505535., Length: 294 bp DNA linear.

Score	Expect	Identities	Gaps	Strand
532 bits(288)	2e-155	292/294(99%)	0/294(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGT
TAGTCAT 60

Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATG
ATTTATGCT 120

Sbjct 61 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTA
CTATTGGT 180

Sbjct 121 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTG
TGCCAACT 240

Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTT
GTG 294

Sbjct 241 .....**T**.....**A**.... 294

The figure appears alignment a sequences analysis for Cox1 gene in *E. vermicularis* local isolates number(5) that recorded in NCBI-BLAST.

***E. vermicularis* isolate IQ-No.6 cytochrome c oxidase subunit I (COX1) gene, partial cds; mitochondrial**

Sequence ID: MZ505536.1, Length: 296 bp DNA linear.

Score	Expect	Identities	Gaps	Strand
536 bits(290)	1e-156	294/296(99%)	0/296(0%)	Plus/Plus

Query 1
TTTTTTGGTCATCCTGAGGTTTATATTCTTATTTTGCCTGCTTTTGGGATTGT
TAGTCAT 60

Sbjct 1 60

Query 61
AGAATTTTGTGTTTAACTGGTAAAAAGGAGGTGTTTGGTCATTTGGGTATG
ATTTATGCT 120

Sbjct 61 .....**C**..... 120

Query 121
ATTATTTCTATTGGTTTAATTGGTAGGGTAGTATGGGGTCATCATATGTTTA
CTATTGGT 180

Sbjct 121 .....**A**..... 180

Query 181
TTTGATATAAGAACACGTTTGTATTTTATGGTTGCTACTATAATTATTGCTG
TGCCAACT 240

Sbjct 181 240

Query 241
GGGGTAAAGGTTTTTAGTTGGTTGTTAACTTTGATAGGGGGACGTTTAGTT
GTGCA 296

Sbjct 241 296

The figure appears alignment a sequences analysis for Cox1 gene in *E. vermicularis* local isolates number(6) that recorded in NCBI-BLAST.

References

Abd-El-Latif, M. E. Z. (2018). The Prevalence of Parasitic Infection of *Entamoeba histolytica, Giardia lambilia* and *Enterobius vermicularis* in Kilowa Governorate, Saudia Arbia. International Journal of Science and Research (IJSR), 8. (4): 1523-1527.

Afrakhteh, N.;Marhaba, Z.; Mahdavi, S.A.; Garoosian, S.; Mirnezhad, R.;Eshkevar Vakili, M.;Ahmadi Shahrajl H.; Javadian, B. and Rezaei, R.,(2015). Prevalence of *Enterobius vermicularis* among Kindergartens and Preschool Children in Mazandaran Province, North of Iran. J. Parasit. Dis., 39(2): 1–3.

Ahmed, M. U.; Bilal, M.; Anis, K.; Khan, A. M.; Fatima, K.; Ahmed, I.; Khatri, A. M. and Rehman, S. (2015). The Frequency of *Enterobius Vermicularis* Infections in Patients Diagnosed With Acute Appendicitis in Pakistan. Global Journal of Health Science, 7. (5): 196- 201.

Ahmed, N. M. ; Al-Niaeemi, B. H.and Dawood, M. H.(2020). Epidemiological and clinical aspects about the endemic intestinal parasites in reviewers of Mosul General hospitals and healthcare centers / Nineveh governorate- Iraq. Eur.Asian Journal of BioSciences Eurasia J. Biosci. 14, 6037-6043.

Al-Saqur; I. M.; Al-Warid, H. S.; Al-Qaisi, A. Q. and Al-Bahadely, H. S.(2020). Prevalence of Gastrointestinal Parasites in Iraq during 2015 . Science and Technology Conf. Proc. 2290, 020005-1–020005-6.

Al-Saqur, I. M.; Al-Warid, H. S. and Albahadely, H. S. (2016). The prevalence of some gastrointestinal nematodes and cestodes in Iraqis. Asian Biomedicine, 10. (1): 61–66.

Albonico, M. ;Becker, S.L.; Odermatt, P.; Angheben, A. ; Anselmi, M.; Amor ,A.;Barda, B.; Buonfrate, D.;Cooper, P.;Gétaz, L.; Keiser, J.; Khieu, V.; Montresor, A.; Muñoz, J. ;Requena-Méndez, A. ;Savioli, L. ;Speare, R. Steinmann, P.; Lieshout, L.; Utzinger, J. and Bisoffi, Z.(2016). An International Network to Improve Diagnostics and Access to Treatment for Strongyloidiasis Control. PLoS Negl. Trop. Dis.J., Vol.10(9).p:1-12.

Al-Daoody, A. A. K. and Al-Bazzaz, E. N. H.(2020). Impact of *Enterobius vermicularis* infection on biochemical parameters in the blood of children in Erbil Province, Iraq . BMC Infectious Diseases J. Vol. 20(336).p:1-6.

Al-Esawi , N. N. S. (2010). Assessment of the activity of Bentonite clay in *Ascaridia galli* and *Enterobius vermicularis* infection and its relationship with Enuresis phenomena among children in Babylon province. M.Sc. Thesis . College of Science - University of Babylon,90pp. (In Arabic).

Alhanout, K.; Malesinki, S.; Vidal, N.; Peyrot, V.; Rolain, J. M. and Brunel, J. M.(2010). New insights into the antibacterial mechanism of action of squalamine .J. Antimicrob. Chemother. 65, 1688–1693 .

Alharbi, R. A.; Alwajeeh, T. S.; Assabri, A. M.; Almalki, S. S. R.; Alruwetei, A. and Azazy, A. A. (2019). Intestinal parasites and *schistosome* infections among students with special reference to praziquantel efficacy in patients with schistosomosis in Hajjah governorate, Yemen. Annals of Parasitology, 65. (3): 217-223.

Al-Hilfi, A. A. A. ; Abu-Mejdad , N. M.J.A. and Minati ,M. H .(2021). Effect of *Citrus Limon* and *Mentha piperita* oil on the *Balantidium coli* in Vitro. Annals of R.S.C.B., Vol. 25, Issue 4, , Pages. 11392 – 11397.

AL-Ibrahimi ,M.H.M (2019). The prevalence of pinworm *Enterobius vermicularis* among children and the relationship between appendicitis and nocturnal enuresis in Al Diwaniyah province. Master of Science in Biology. College of Science. University of AL-Qadisiya; 106 pp.(In Arabic).

AL-Jabri, N. N. and Hossain, M. A.(2016). Chemical composition and antimicrobial potency of locally grown lemon essential oil against selected bacterial strains. King Saud University –Science J .72,p:1-7.

AL-Jaf . E.E.H; Rahi,A.S.A. and Tofah.J.A.R(2018). Comparative Study between Molecular and Classical Methods for Defection *Enterobius vermicularis* inWasit

Province . Master of Science in Biology- Zoology. College of Science,University of Wasit ; 44 P.p.

Al-kabee . R . M . R. ; Dawood . K . A and Al-Essami , A . H . (2014). Epidemiological, Immunological and molecular study of *Enterobius vermicularis* in Al-Diawnia Governorate , College of Medicine/ Al -Qadisiya University , Master of Science in Medical Microbiology.

Kang, S.; Sultana, T.; Eom, K. S.; Park, Y. C.; Soonthornpong, N.; Nadler, S. A. and Park, J. K. (2009). The mitochondrial genome sequence of *Enterobius vermicularis* (Nematoda: Oxyurida) – An idiosyncratic gene order and phylogenetic information for chromadorean nematodes. Gene. 429: 87–97.

Al-Kinany , A . M . H . (2020) . Morphological and Molecular Study of *Enterobius Vermicularis* in Wasit Province . Master thesis . College of Science - Wasit University.78pp.

Al-Morshidy ,K.A.(2007). Prevalence of intestinal pathogenic parasites infections in Hilla city, Babylon province, Iraq . J. Babylon Univ. Pur. Appl. Sci., Vol.14, No., 1, 2007: 179-185.

Al-Madhi , S. A. S. (2016). Compratitive study of mitochondrial genome of *Raillietina* spp. Cestoda in pigeon and domestic chicken , and the study of watery extract of *Dainthus caryophyllus* in vitro. Master in Biology, Faculty of Education for Girls, University of Kufa:109p.

Almeida , A. C. A. D. ; de-Faria , F. M.; Dunder R. J., Manzo, L. P. B.; Souza-Brito, A. R. M. and Luiz-Ferreira, A .(2017). Recent Trends in Pharmacological Activity of Alkaloids in Animal Colitis: Potential Use for Inflammatory Bowel Disease. V. 2017, 24 pages.

Al-Minsouri , N . A . A . (1995) . Effect of different extracts from a plant *Ibiceila lutea* In the life performance of the White Fly *Bemisa tabaci* Doctoral thesis philosophy of the Faculty of Science, Basra University.

Al-Mozan,H.D.and Dakhil, K.M.(2019). Prevalence of Parasites in Fresh Vegetables from Two Regions of Thi-Qar Province, Iraq Journal of Pure and Applied Microbiology.13(.2).49.

Al-Nakkas, E. M.; Al-Mutar, M. S.; Shweiki, H. M.; Sharma, P. N. and Rihan, S. (2004). Parasitic infections in Kuwait : A study based on Primary Care Centers. Middle East Journal of Family Medicine, 3. (3): 4–10.

Al-Quraishi, M.Ch. (2006). The relationship between the infestation with *Enterobius vermicularis* and Enuresis in children, Med. J. Babylon, 3 (1-2): 164-168.

Al-Quraishi, K . K . (2020) . Genotyping study to *Enterobius vermicularis* parasite and some pathogenic bacterial infection and their relationship with urinary tract infection among female in Wasit Province. Master thesis . College of Science - Wasit University. 103pp.

Al-Rawi, K.M. and Khalaf-Allah, A.M.(2000). Design and analysis of agriculture experiments. Book house for publishing and printing , Mosul University.

Al‑Rifai, R. H. ; Loney, T. ; Sheek‑Hussein, M.; Zoughbor, S.; Ajab, S. ; Olanda, M.; Al‑Rasbi, Z.(2020). Prevalence of, and Factors Associated with Intestinal Parasites in Multinational Expatriate Workers in Al Ain City, United Arab Emirates: An Occupational Cross‑Sectional Study. Journal of Immigrant and Minority Health . 22,p:359–374.

Alrubei , H . M . (1999) . The effect of Datura innoxa plant extract in some sides of life performance for house fly *Musca domestica* .Doctoral thesis philosophy of the Faculty of Science, Babylon University.

Al-Samarai , R. M . K .(2020) . Epidemiological study and molecular characterization of pinworm(*Enterobius vermicular*)among children at Thi-Qar governorate . Ph.D. thesis. College of Science at University of Kufa .108pp.

Al-Shadood , H. A. S. (2015) . Study the association between *Enterobius vermicularis* infection and enuresis among children in Al- Najaf city. AL-Qadisiya Journal of Veterinary Medicine Science, 14.(1): 104–108.

Alsubaie, A. S. R .; Azazy , A. A.; Omer, E. O.; Al-shibani, L. A.; Al- Mekhlafi, A. Q. and Al-Khawlani, F. A. (2016). Pattern of parasitic infections as public health problem among school children: A comparative study between rural and urban areas. Journal of Taibah University Medical Sciences, 11. (1): 13-18.

Al-Taei, A. H. O. (2019). The prevalence of intestinal parasite among the attending peoples to Al-Hashimyah hospitals for seven years, Babylon province, Iraq. Journal of Physics: Conference Series, 1294. (6): 1-9.

Alwaily, A. G. A.; Alomashi, G. B. and Al-Obiedy, O. T. (2019). The association between helminthes infestation and IBD in the context of NOD2/CARD15 gene polymorphism and serum levels of IL1Beta and IL10. University of Al-Qadisiyah:1-104.

Anand ,U.; Jacobo-Herrera, N.;Altemimi ,A. and Lakhssassi, N. (2019). A Comprehensive Review on Medicinal Plants as Antimicrobial Therapeutics: Potential Avenues of Biocompatible Drug Discovery. Metabolites. 1;9(11):258 .

Anku,w.w.; Mamo, M. and Govender ,P. (2017). Phenolic Compounds in Water: Sources, Reactivity, Toxicity and Treatment Methods. In: Soto-Hernandez, M.; Palma-Tenango, M. and Garcia-Mateos, M. R. (ed.) Phenolic Compounds-Natural Sources, Importance and Applications. South Africa.InTechOpen.

Antherden , L. M. (1969) . Bently and drivers textbook of pharmaceutical chemistry oxford university press, London, 8th edn., 916 PP .

Asires, A.; Wubie, M. and Reta, A. (2019). Prevalence and associated factors of intestinal parasitic infections among food handlers at prison, East and West Gojjam, Ethiopia. BMC Research Notes, 12. (1): 1-8.

Avila, A.S.; Zambom, M.A.; Faccenda, A.; Fischer, M.L.; Anschau, F.A.; Venturini, T.; Tinini, R.C.R.; Dessbesell, J.G. and Faciola, A.P.(2020). Effects of black wattle

(Acacia mearnsii) condensed tannins on intake, protozoa population, ruminal fermentation, and nutrient digestibility in jersey steers. Animals J., 10(6) ,P:1-12.

Aziz, A.; Raju, G. S.; Das, A.; Ahmed, J. and Moghal ,M. R. (2014). Evaluation of *In vitro* Anthelmintic Activity, Total Phenolic Content and Cytotoxic Activity of *Crinum latifolium* L.(Family: Amaryllidaceae) . Advanced Pharmaceutical Bulletin, 2014, 4(1), 15-19.

Azwanida, N.N. (2015). A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Medicinal and Aromatic Plants, 4, 196.

Babat, S. O.; Sirekbasan, S.; Macin, S.; Kariptas, E. and Polat, E. (2018). Diagnostics of intestinal parasites by light microscopy among the population of children between the ages of 4-12 in eastern Turkey. Tropical Biomedicine, 35. (4): 1087-1091.

Bahrami, F.; Haghigh, A,; Zamini, G.; Erfan, M. B. K. and Azargashb, E. (2018). Prevalence and associated risk factors of intestinal parasitic infections in Kurdistan province, northwest Iran. Cogent Medicine, 5.(15): 1-14.

Barker, N. (2014). Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. Nat. Rev. Mol. Cell Biol. 15, 19–33.

Bergquist , R. ; Utzinger , J. and Keiser, J. (2017). Controlling schistosomiasis with praziquantel : how much longer without a viable alternative? Infect. Dis. Poverty.;6(1):74 .

Beyhan , Y . E . ; Yilmaz , H. and Hokelek , M . (2016) . Effects of acetic acid on the viability of *Ascaris lumbricoides* eggs is vinegar reliable enough to clean the vegetables.Saudi Med. J. 2016; Vol. 37 (3): 288-292.

Bhambhani, S.; Kondhare, K.R. and Giri, A.P. (2021). Diversity in Chemical Structures and Biological Properties of Plant Alkaloids. Molecules, 26, 3374.P: 1of 29.

Bharti, B.; Bharti, S. and Khurana, S. (2018). Worm Infestation: Diagnosis, Treatment and Prevention. Indian Journal of Pediatrics, 85.(11): 1017–1024.

Boberek, J.M. ; Stach, J. and Good, L. (2010). Genetic evidence for inhibition of bacterial division protein FtsZ by berberine. PLoS One J.,5(10)P:1-9.

Boncan ,D. A. T. ; Tsang ,S. S.K. ; Li, C.; Lee ,I. H.T. ; Lam ,H.–M.; Chan,T.–F.and Hui, J. H.L. (2020). Terpenes and Terpenoids in Plants: Interactions with Environment and Insects. International Journal of Int. J. Mol. Sci., 21, 7382.

Bryce, J. R. and Poelma, P. L .(1998). Bacteriological Analytical Manual, 8th Edition, Revision A .

Budak, N.; H., Aykin E.; Seydim, A.C.;Greene, A. K. and Guzel-Seydim, Z.B.(2014). Functional Properties of Vinegar .Vol. 79: (5) Journal of Food Science.757-764.

Çakırlar, F.K.; Yıldız, S.;Aydoğan, O.; Özdemir, S.; Çolakoğlu,A.; Taner, Z.(2019). The Investigation of Intestinal Parasites and VRE Carriage in Children Studying in Schools with Different Socio-Economic Levels in İstanbul, Turkey. Experimed , 9(1): 7-10.

Chai, J. Y.; Yang, S. K.; Kim, J. W.; Choi, S. L.; Song, G. Y.; Jung, B. K.; Kim, M. J.; Cho, J.; Kim, D. G.; Sohn, W. M.; Jeoung,H. G.; Cho, S.; Park, J. B.; Hong, S.; Htoon, T. T. and Tin, H. H. (2015). High Prevalence of *Enterobius vermicularis* Infection among Schoolchildren in Three Townships around Yangon, Myanmar. Korean Journal Parasitol, 53. (6): 771-775.

Chen, H. Y.; Chen, T., Giudici, P. and Chen, F. S. (2016). Vinegar functions on health: Constituents, sources, and formation mechanisms. Comprehensive Reviews in Food Science and Food Safety, 15, 1124–1138.

Chen, K. Y.; Yen, C. M.; Hwang, K. P. and Wang, L. C. (2018). *Enterobius vermicularis* infection and its risk factors among pre-school children in Taipei, Taiwan. Journal of Microbiology, Immunology and Infection, 51. (4): 559–564.

Collee , J. G. ; Frasser , A. G. ; Marmion , B. P. and Simmon, A. S. (1996) . Parctical medical microbiology. Chunchill living stone . Elsevier.UK : 496 pp .

Cowan, N. ;Meier C. ; Neodo, A. and Keiser, J. (2017). Exposure of *Heligmosomoides polygyrus* and *Trichuris muris t*o Albendazole , albendazole sulfoxide, mebendazole and oxantel pamoate *in vitro* and *in vivo* to elucidate the pathway of drug entry into these gastrointestinal nematodes. International Journal for Parasitology,Vol. 7.P:159-173.

Cushnie, T. T., Cushnie, B., and Lamb, A. J. (2014). Alkaloids: An overviewof their antibacterial, antibiotic-enhancing and antivirulence activities. Int. J.Antimicrob. Agents 44, 377–386. doi: 10.1016/j.ijantimicag.2014.06.001

Dafalla, A. I. A.; Almuhairil, S. A. S. O. ; AlHosani, M. H. J.; Mohamed, M. Y.; Alkous, M. I. A.; AlAzzawi, M. A.; Abakar, A. D.; Nour, B. Y. M.; Hasan, H.; Aboodeh, R.O. and Elbakari, A. (2017). Intestinal parasitic infections among expatriate workers in various occupations in Sharjah, United Arab Emirates. Journal of the Saopaulo Institute of Tropical Medicine. 59: 1–7.

Dahal, T. and Maharjan, M.(2015). Pinworm (*Enterobius vermicularis*) Infection in Children of Barbhanjyang VDC, Tanahun District. Journal of Institute of Science and Technology,Vol.20(2),p: 18-21.

De-Groot , A.C. and Schmidt, E. (2016a). Essential oils, part I: Introduction. Dermatitis;27(2):39–42.

De-Groot , A.C. and Schmidt, E. (2016c). Essential Oils, Part III: Chemical Composition. Dermatitis Limonene. ;27(4):161-9 .

De-Groot , A.C. and Schmidt , E.(2016b). Essential oils, part II: general aspects. Dermatitis;27 (2):43–49.

Delgado, R., Delgado, M.G., Bastin-Héline, L., Glavic, A., O'Day, P.M. and Bacigalupo, J. (2019). Light-Induced Opening of the TRP Channel in Isolated Membrane Patches Excised from Photosensitive Microvilli from *Drosophila* Photoreceptors. Neuroscience journal,396,p: 66--72.

Despommier, D. D.; Griffin, D. O.; Gwadz, R. W.; Hotez, P. J. and Knirsch, C. A. (2017). Parasitic Diseases (6th Edition). Sentinel printing USA, NY:201-205.

Dezsényi, B.; Sárközi, L.; Kaiser, L.; Tárkányi, K.; Nikolova, R.and Belics, Z. (2018). Gynecological and obstetrical aspects of E. vermicularis infection. Acta Microbiologica et Immunologica Hungarica, 65. (4): 459–465.

Dick, L. and Hannay, J. (2017). *Enterobius vermicularis* presentation during laparoscopic cholecystectomy. Journal of Surgical Case Reports. 2017; (1)p: 239 .

Dudlová, A.; Juriš, P.; Jarčuška, P.; Vasilková, Z.; Vargová, V.; Sumková, M. and Krčméry, V. (2018). The incidence of pinworm (*Enterobius vermicularis*) in pre-school and school aged children in the Eastern Slovakia. Helminthologia, 55.(4): 275–280.

Eguchi, R.; Ono, N. ; Morita ,A. H.; Katsuragi,T.; Nakamura, S.; Huang, M.; Altaf-Ul-Amin, M. and Kanaya, S. (2019). Classification of alkaloids according to the starting substances of their biosynthetic pathways using graph convolutional neural networks . BMC Bioinformatics.Vol. 20(380),p:1-13 .

El-Kutry, M.S. and Sopeah H. R. A. (2020). Impact of Intake a Sidr (*Zizyphus Spina-Christi L.*) Extract on *E. vermicularis* Infection for Children Vol. 13(1), p. 359-366.

Elija, K. ; Vaishali, A. ; Rasika, T.; Sucheta, G. ; Nirmala D. and Rajashree, K. (2017). Spectroscopic determination of total phenol and flavonoid contents of *Citrus limon* peel from north eastern region of India. Journal of Drug Delivery & Therapeutics. 7(1):21-24.

Elija, K. ; Vaishali, A. ; Rasika, T.; Sucheta, G. ; Nirmala D. and Rajashree, K. (2017). Spectroscopic determination of total phenol and flavonoid contents of *Citrus limon* peel from north eastern region of India. Journal of Drug Delivery & Therapeutics.; 7(1):21-24.

Emile,N.; Bosco ,N. J. and Karine,B.(2013). Prevalence of intestinal parasitic infections and associated risk factors among Kigali Institute of Education students in Kigali, Rwanda Tropical Biomedicine.Vol. 30(4): 718–726 .

Eraky, M. A.; Rashed, S. M. and Naser, M. E. S. (2014). Parasitic Contamination of Commonly Consumed Fresh Leafy Vegetables in Benha, Egypt. Journal of Parasitology Research. 1-7.

Erasto, P.and Viljoen,A. M.(2008). Limonene-a Review:Biosynthetic, ecological and pharmacological relevance.natural product communications .V.3(7) p:1193-1202.

Etemadi, S. and Hosniehoseini (2021). Study of the role of IQ in children with *Enterobius vermicularis* infestation compared to healthy children. Open J. Pediatr Child Health 6(1): 043-045 .

Fan, C.–K. ; Chuang ,T.–W. ; Huang, Y. -C.; Yin, A. –W.; Chou, C. -M. ; Yu-Ting Hsu,; Kios, R.; Hsu, S.-L.; Wang, Y. -T.; Wu, M. -S.; Lin, J. -W.; Briand, K. and Tu,C. –Y.(2019). *Enterobius vermicularis* infection: prevalence and risk factors among preschool children in kindergarten in the capital area, Republic of the Marshall Islands. BMC Infectious Diseases , 19:536,p: 2 – 7.

Ferreira, L. F. ; Reinhard, K. J. and Araújo, A.(2014). Foundations of Paleoparasitology. Journal of International Federation for Tropical Medicine ,p:484 .

Ferrero, M. R.; Röser, D.; Nielsen, H. V.; Olsen, A. and Nejsum,P. (2013). Genetic variation in mitochondrial DNA among *Enterobius vermicularis* in Denmark. Parasitology, 140.(1): 109–114.

Foitová, I. ; Civáňová, K. ; Baruš, V. and Nurcahyo,W.(2014). Phylogenetic relationships between pinworms(Nematoda: *Enterobiinae*) parasitizing the critically endangered orang-utan, according to the characterization of molecular genomic and mitochondrial markers. Journal of Parasitol Res.

Friesen, J.; Bergmann, C.; Neuber, R.; Fuhrmann, J.; Wenzel, T.; Durst, A.; Müller, M. and Ignatius, R. (2019). Detection of *Enterobius vermicularis* in greater Berlin,

2007–2017: seasonality and increased frequency of detection. European Journal of Clinical Microbiology & Infectious Diseases: 1-5.

Garcia, L. S.; Arrowood, M.; Kokoskin, E.; Paltridge, P. P.; Pillai, D. R.; Procop,G. W.; Ryan, N.; Shimizu, R. Y. and Visvesvara, G. (2018). Laboratory Diagnosis of Parasites from the Gastrointestinal Tract. Clinical Microbiology Reviews, 31.(1): 1-81.

Giada , M. L. R .(2013). Food Phenolic Compounds: Main Classes, Sources and Their Antioxidant Power. In: Moralez-González, J. A. Oxidative Stress and Chronic Degenerative Diseases: A role for antioxidants, United Kingdom, IntechOpen, London. .pp:514.

Gil, N.Y.; Gwon, H.M.; Yeo, S. H. and Kim, S.Y. (2020). Metabolite Profile and Immunomodulatory Properties of Bellflower Root Vinegar Produced Using Acetobacter pasteurianus. Foods,J.;9(8):1063.

Gillespie, S. H. and Pearson, R. D.(2001). Principles and practice of clinical parasitology. Library of Congress .USA:573-574.

González-Mas, M.C.; Rambla, J.L.;López-Gresa, M.P.; Blázquez, M.A. and Granell , A. (2019). Volatile Compounds in *Citrus* Essential Oils: A Comprehensive Review. Front. Plant Sci. 10:12.

Gunaratna, P.S.G; Dempsey, S.; Caylee, M.R.S. and Britton , P. N .(2020). DIAGNOSIS OF *ENTEROBIUS VERMICULARIS* INFESTATIONS. Journal of Pediatrics and Child Health 56 , 1993–2002.

Gupta , G .; Agarwal, U.; Kaur , H. ; Kumar,N. R. and Gupta, P.(2017). Aphicidal effects of terpenoids present in *Citrus limon* on *Macrosiphum roseiformis* and two generalist insect predators. of Asia-Pacific Entomology J. V. 20(4), P: 1087-1095.

Gutiérrez-Grijalva, E.P. ; Lopez-Martinez, L. X.; Contreras-Angulo, L.A.; Elizalde-Romero, C. A. and Heredia ,J.B. (2020). Plant Alkaloids: Structures and Bioactive in Plant-derived Bioactives. M. K. Swamy. Springer Nature Singapore. Pte Ltd. P:108.

Hagh, V. R. H.; Oskouei, M. M.; Bazmani, A.; Miahipour, A. and Mirsamadi, N. (2014). Genetic classification and differentiation of *Enterobius vermicularis* based on mitochondrial cytochrome c oxidase [cox1) in Northwest of Iran. Journal of Pure and Applied Microbiology, 8.(5): 3995–3999.

Hama, A. A. and Rahemo, I. F. (2014). Intestinal Parasitosis in Relation To Haemoglobin Concentration Among primary schoolchildren in Erbil province Kurdistan-Iraq. International Science Journal, 1.(1): 96–99.

Hamarsheh, O. (2021). Epidemiology of Enterobiasis in Palestine. Al-Quds J. Acad. Res. 1(1),p:63-67.

Hamdona, S. M.; Lubbad, A. M. and Al-Hindi, A. I. (2013). Histopathological study of *Enterobius vermicularis* among appendicitis patients in Gaza strip. J Parasit Dis, 40.(1):176–183.

Hamid, M.(2019). Effect of *Enterobius vermicularis* parasite on IgE antibody levels among children in Al- Diwaniyah City, middle Iraq. Al-Qadisiyah Journal Of Pure Science Vol. 24, No. 4, pp. 51 –55 .

Harborne , J. B. (1984) . Phytochemical methods : Aguide to modern techniques of plant analysis . 2nd ed ., chapmant and Hall , London , 288 pp.

Harborne, J. B. (1973) . Phytochemical methods chapman and Hall. London, P. 612 .

Hechenbleikner, E. M. and Fascrs, J. A. M. M. F. (2015). Parasitic colitis. Clinics in Colon and Rectal Surgery, 28.(2): 79–86.

Hegedusova, E.; Brejova, B.; Tomaska, L.; Sipiczki, M. and Nosek, J. (2014) .Mitochondrial genome of the *basidiomycetous* yeast *Jaminaea angkorensis*. Curr. Genet. 60:49–59.

Hemke, J.; Rasane , P.; Kaur, S.; Kumbhar, P. and Singh, J.(2019). Vinegar: a traditional functional food.THINK INDIA J. Vol-22-Issue-34.581-629 .

Hernández, P. C.; Morales, L.; Olaya, J. C.; Sarmiento, D.; Jaramillo, J. F.; Ordoñez, G. A. and Sánchez, L. K. (2019). Intestinal parasitic infections and associated factors in children of three rural schools in Colombia. A cross-sectional study. Plos One, 14.(7): 1-19.

Hong, S.-T. (2018). Albendazole and Praziquantel: Review and Safety Monitoring in Korea. Infect Chemother;50(1):1-10.

Horton, J. (2020). Albendazole: A review of anthelmintic efficacy and safety in humans. Parasitology, 121(S1), S113-S132 .

Houpt,E. R. and Chaudhry, O.(2009). Protozoa and helminthic infections.In: Waldman, S. A.and Terzic,A.(eds.) Pharmacology and Therapeutics Principles to Practice. Pharmacology and Therapeutics,W.B. Saunders,1st.ed., 1378pp.

Huang ,S. ; Liu, X. ; Xiong , B. ; Qiu , X. ; Sun ,G. ; Wang, X.; Zhang ,X. ; Dong, Z. and Wang, Z. (2019) . Variation in limonin and nomilin content in *Citrus* fruits of eight varieties determined by modified HPLC Food Sci. Biotechnol. 28(3):641–647.

Hudson , L. and Hay , F. C. (1984) . Practical immunology. 3rd edn., Black well. Scientific Publication.

Hugot,J.–P.;Gardner, S.L.;Borba,V.;Araujo,P.;Leles, Da-Rosa, D.;Á.A. S. Dutra, J., Ferreira , L. F. and Araújo, A.(2014). Discovery of a 240 million year old nematode parasite egg in a cynodont coprolite sheds light on the early origin of pinworms in vertebrates. Parasites & Vectors,J.7:486P:2-8.

Hussein, J. N. and Meerkhan, A. A. (2019). The Incidence Of Intestinal Parasites Among Children In Hivi Pediatric Hospital, Duhok, Iraq. Science Journal of University of Zakho.Science Journal of University of Zakho, 7.(1): 1–4.

Hussein, Z. A. (2015). Prevalence *Enterobius vermicularis* infection in children and relationship with nocturnal enuresis in Najaf Province. Journal of Kerbala University, 13. (3): 81–86.

Ibáñez, M. D.; Sanchez-Ballester, N.M. and Blázquez ,M. A.(2020). Encapsulated Limonene: A Pleasant Lemon-Like Aroma with Promising Application in the Agri-Food Industry. A Review. Molecules, J., 25, 2598.

Inabo , H. I. and Fathuddin , M. M. (2011) . Antitrypanosomal potentials of ethanolic leaf extracts of *Punica granatum* against *Trypanosoma brucei brucei* infection. Bay. J., Pu. App. Sci., 4(2):35-40.

Jackie ; Lee, C. and Toyama, A.(2020). Shimadzu's Fundamental Guide to Gas Chromatography Mass Spectrometry (GCMS). Shimadzu's corporation. first ed.p:87.

Jaegera, R. and Cuny, E.(2016). Terpenoids with Special Pharmacological Significance: A Review. Natural Product Communications Vol. 11 (9),p:1373-1390..2016Vol. 11- 30

Jamaludin, M.A. ; Amin, A. ; Fadzlillah, N. A. ;Kartika, B. ;Othman, R. ;Sani, S. and Ramli, A.(2017). Study on physiochemical properties and the halalness of commercially marketed vinegar in Malaysia. International Food Research Journal ,Vol.24 p: S428-S435

Jamshidi-Kia , F.; Lorigooini, Z. and Amini-Khoei, H.(2018) . Medicinal plants: Past history and future perspective . Journal of Herbmed Pharmacology , 7(1): 1-7.

Jaradat ,N.; Adwan ,L.; Kaibni, S.; Shraim, N. and Zaid ,A. N.(2016). Chemical composition, anthelmintic,antibacterial and antioxidant effects of *Thymus bovei* essential oil. BMC Complementary and Alternative Medicine 16:418,p:1-7.

Jatsa, H. B. ; Femoe , U. M.; Daniel, J. N.; Tombe,S. T. ; L. N. Mbolang; Nkondo ,E. T.; Tchuente, L. –A. T.; Dimo, T. and Kamtchouing, P. (2018). Efficacy of *Sida pilosa Retz* aqueous extract against *Schistosoma mansoni* –induced granulomatous inflammation in the liver and the intestine of mice: histomorphometry and gastrointestinal motility evaluation. BMC Complementary and Alternative Medicine 18:247.

Johansson, J.; Ignatova, S. and Ekstedt, M. (2013). Pinworm infestation mimicking Crohns' disease. Case Reports in Gastrointestinal Medicine.1-4.

Jones.M. (2019). Gas Chromatography-Mass Spectrometry. CAS of American Chemical Society.p:4.

Kadir, M. A. A. and Amin, O. M. (2011). Prevalence of enterobiasis (*Enterobius vermicularis*) and its impacts on children in Kalar Town / Sulaimania- Iraq . Tikrit Medical Journal , 17. (2): 67-77.

Kandi, V.; Vaish, R.; Palange, P. and Koka, S. S. (2019). *Enterobius Vermicularis* : Does it Invade Central Nervous System ? American Journal of Infectious Diseases and Microbiology. 7: 8–12.

Kara, S. S. and Volkan, B. (2018). Differences Between The Children Infected with *Enterobius Vermicularis* and Taenia Saginata. Sakarya Med Journal, 8.(2): 272-278.

Karaman, U.; Kiran, T. and Colak, C. (2014). The Levels of Adenosine Deaminase (ADA) in the Serum of *Enterobius Vermicularis* Positive Patients. Medicine Science, 3. (4):1648–1654.

Kaskooz, R. A. .(2019). Essential Oil Analysis by GC-MS and Analgesic Activity of *Lippia citriodora* and Citrus limon .Journal of Essential Oil Bearing Plants. Vol: 22(1). Pages: 273-281.

Kern, E.M.A.; Kim, T. and Park, J-K .(2020) . The Mitochondrial Genome in Nematode Phylogenetics . Journal of Front. Ecol. Evol.Vol. 8(250),p:1-8.

Khadka, K. and Maharjan, M. (2018). Pinworm (*Enterobius vermicularis*) Infection among Primary Level Government School Children of Chhampi, Lalitpur District, Nepal. National Journal of Health Sciences, 3. (2): 46–50.

Khan , W.; Panhwar , W. A. ; Mehmood , S. A. ; Ahmed , S.; Ahmedd, M. S. ; Khane , N. ; Khana, M. M. ; Akramc, W.; Ullahd, S. and Imranc.(2020). Pinworm infection in school children of four districts of Malakand region, Khyber Pakhtunkhwa, Pakistan . Brazilian Journal of Biology, vol.(82),p:1-6.

Khatiwora, E.(2018). *Citrus limon* leaves from north-eastern India: a potential source of anthelmintic agent against *Eicinia foetida*, Journal of Drug Delivery and Therapeutics.; 8(3):55-57.

Khoshnood , S . ; Saki , J . ; Rafiei , A . and Alizadeh , K . (2015) . Prevalence of Intestinal Parasitic Infections Among People in Baghmalek During 2013 – 2014. Jen. J. Hea. Res. 6(2): 24792.

Kim, T.; Kern, E.; Park, C.; Nadler, S. A.; Bae, Y. J.; and Park, J.-K. (2018). The bipartite mitochondrial genome of *Ruizia karukerae* (Rhigonematomorpha, Nematoda). Sci. Rep. 8:7482.

Klimek-Szczykutowicz, M. ; Szopa, A. and Ekiert ,H.(2020).*Citrus limon* (Lemon) Phenomenon—A Review of the Chemistry, Pharmacological Properties, Applications in the Modern Pharmaceutical, Food, and Cosmetics Industries, and Biotechnological Studies.Plants, J., 9(119)P:24 .

Kubiak, K.; Dzika, E. and Paukszto, L. (2017). Enterobiasis epidemiology and molecular characterization of *Enterobius vermicularis* in healthy children in north-eastern Poland. Helminthologia, 54.(4): 284-291.

Kuo, P. –C. ; Liao, Y. -R. ; Hung, H. –Y. ; Chuang ,C. –W.; Hwang,T. -L.; Huang ,S. – C. ; Shiao ,Y. -J. ; Kuo ,D. -H. and Wu, T. –S.(2017). Anti-Inflammatory and Neuroprotective Constituents from the Peels of *Citrus grandis*. Journal of Molecules, 22, 967.

Kurek, J. (2019). Alkaloids - Their Importance in Nature and Human Life. IntechOpen. London.

Lalthanpuii, P.B. and Lalchhandama, K.(2020). Phytochemical analysis and *In vitro* anthelmintic activity of *Imperata cylindrica* underground parts. BMC Complement Med Ther. Nov 6;20(1):332.

Laoraksawong, P.; Suntaraluk, A.; Kongnil, W.; Pongpanitanont, P. and Janwan, P. (2020). Prevalence of Soil-Transmitted Helminth Infections and Associated Risk

Factors among Schoolchildren in Nakhon Si Thammarat, Thailand. Iran J. Parasitol.;15(3):440-445 .

Lattanzio, V. (2013). Phenolic compounds: Introduction. In Natural Products; Springer: Berlin/Heidelberg, Germany,; pp. 1543–1580.

Leblanc, M.; Berry, K.; Graciano, S.; Becker, B. and Reuter, J. D. (2014). False-positive results after environmental pinworm pcr testing due to rhabditid nematodes in corncob bedding. Journal of the American Association for Laboratory Animal Science, 53.(6): 717–724.

Lee, K. J.; Bae Y.; Kim, H.; Deung, Y. K. and Yong, T. S. (2002). Status of Intestinal Parasites Infection among Primary School Children in Kampongcham Korean, J. Para.; 40 (3):153-155.

Li, H.-M. ; Zhou, C. -H. ; Li, Z. –S.; Deng, Z. –H. ; Ruan, C. -W.; Zhang, Q. –M. ; Zhu, T. –J. ; Xu , L. –Q. and Chen, Y. –D.(2015). Risk factors for *Enterobius vermicularis* infection in children in Gaozhou, Guangdong, China Infectious Diseases of Poverty.4:28.

Lin, X. ; Stur, E. and Ekrem, T. (2015). Exploring genetic dive.rgence in a species-rich insect genus using 2790 DNA barcodes. PLoS One.;10:e0138993.

Lino, M.; Lelesb, D.; Peñac, A. P. and Vinaud, M. C.(2018). First description of *Enterobius vermicularis* egg in a coprolite dated from the pre-contact in Brazil.Journal of Archaeological Science: 17,P:1–6.

Luzón-Quintana, L.M.; Castro, R. and Durán-Guerrero, E. (2021). Biotechnological Processes in Fruit Vinegar Production. Foods, 10, 945.p:1-23.

Maaroufi,Z.;Cojean, S. ; Loiseau,P. M.; Yahyaoui, M. ; Agnely F. ; Abderraba, M. and Mekhloufi, G. (2021). *In vitro* antileishmanial potentialities of essential oils from *Citrus limonand Pistacia lentiscus* harvested in Tunisia. Parasitol Res. 120(4):1455-1469 .

Mahato, N. ; Sharma, K. ; Koteswararao, R. ; Sinha, M. ; Baral, E. and Cho, M.H. (2019). *Citrus* essential oils: Extraction, authentication and application in food preservation. Crit. Rev. Food Sci. Nutr.;59(4):611-625 .

Mahmoud , A. E. and Ali , M. M. (2012) . Attenuation the side effects of adriamycin – induced cardiotoxicity and nephrotoxicity in rats by fermented *Punica granatum* (pomegranate) peel extract . Int . J. Res. Pharma . Sci. , 3(1) : 29-37.

Makni,M.; Jemai, R.; Kriaa, W. ; Chtourou, Y. and Fetoui, H. (2018) . *Citrus limon* from Tunisia: Phytochemical and Physicochemical Properties and Biological Activities .Bio Med Research International Vol. 2018, p: 10.

Marrelli, M.(2021). Medicinal Plants: Medicinal Plants. Journal of Plants, 10, 1355 .

McCarthy ,J. S. and Moore, T. A. (2020). Drugs for Helminths . In: Bennett, J. E. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, Ninth Edition, Elsevier, 4th.ed. 555pp.

Mehlhorn, H. (2016). Human Parasites Diagnosis, Treatment, Prevention. Springer Spektrum, Germany: 211-213.

Meletis, G.; Sdravos, C.; Beta, E. and Chatzidimitriou, D. (2019). Moving *Enterobius* larvae inside their eggs. Clinical Microbiology and Infection Journal, 25.(8): 971-972.

Meskin, M. V.; Hamedi, Y.; Heydarihengami, M.; Eftekhar, E.; Shamseddin, J. and Sharifisarasiabi, K. (2019). Intestinal parasitic infections in mental retardation center of Bandar Abbas, southern Iran. Iranian Journal of Parasitology, 14 .(2): 318–325.

Moosazadeh, M.; Abedi, G.; Afshari, M.; Mahdavi, S. A.; Farshidi, F. and Kheradmand, E. (2017). Prevalence of *Enterobius vermicularis* among children in Iran: A systematic review and meta- analysis. Osong Public Health and Research Perspectives, 8.(2): 108– 115.

Motran, C.C.; Silvane, L.; Chiapello, L.S.,; Theumer, M.G.; Ambrosio, L.F.; Volpini, X. ;Celias, D.P. and Cervi, L.(2018). Helminth Infections: Recognition and

Modulation of the Immune Response by Innate Immune Cells. Front Immunol. 4;9:664.

Mukherjee, T.; Sanyal, P.; Deysarkar, S. and Basu, A. (2015). *Enterobius vermicularis* in colon mimicking tubercular ulcers. International Journal of Current Research and Academic Review, 3. (2): 143–146.

Muluye, A. B.; Desta, A. G. ; Abate S. K. and Dano, G. T .(2019). Anti‑malarial activity of the root extract of *Euphorbia abyssinica* (Euphorbiaceae) against *Plasmodium berghei* infection in mice. Malaria Journal . 18:261RESEARCH

Munne, S. ; Parwate, D. ; Ingle, V. and Nagpurkar, V. (2011). Evaluation of the anthelmintic activity of *Citrus limon* juice sacs, Int. J. Pharma. V.2:(2).

Musa,I. S.(2017). Incidence of helminthiasis in humans in Iraq. Karbala International Journal of Modern Science.Vol. 3,p: 267-271.

Nakano, T. ; Okamoto, M.; Ikeda, Y. and Hasegawa, H. (2006) . Mitochondrial cytochrome c oxidase subunit 1 gene and nuclear rDNA regions of *Enterobius vermicularis* parasitic in captive chimpanzees with special reference to its relationship with pinworms in humans. Parasitology Research 100,p: 51–57.

Niaz, S. ; Abdullah; Arif, M.; Zubair, N.; Khan, S. and Zeb, M.A. (2019). Prevalence of *Enterobius vermicularis* in Children of District Mardan, Pakistan. PSM Biol. Res., 4(2): 58-62.

Oba, Y. ;Ôhira, H. ;Murase, Y.; Moriyama, A. and Kumazawa, Y . (2015). Barcoding of Japanese click beetles (Coleoptera, Elateridae). PLoS One.;10:e0116612.

Obaid, H. (2018). *Enterobius vermicularis* infection in relation to appendicitis and fallopian tube obstruction in population of Kirkuk city. Al-Kitab Journal for Pure Sciences, 2. (1): 229–243.

Oliewi ,M. K. and Al-Hamairy ,A. K. (2016). Epidemiological and Diagnostic Study for Diarrheic Parasites (*Entamoeba histolytica, Giardia lamblia,* and *Cryptosporidium* sp.)

Among Diarrheic Infected Patients By Using Multiplex Polymerase Chain Reaction in the Babylon province, Iraq. Res. J. Pha., Bio. and Che. Sci. 7:(1) : 438-447.

Otang, W.M. and Afolayan, A.J. (2016) . Antimicrobial and antioxidant efficacy of *Citrus limon* L. peel extracts used for skin diseases by Xhosa tribe of Amathole District, Eastern Cape, South Africa. S. Afr. J. Bot. v. 102, p:46–49.

Othman, L.; Sleiman, A. and Abdel-Massih, R.M. (2019). Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. Front. Microbiol. J.Volume 10 ; 911.

O**tu-Bassey, I. B.; Useh, M. F. and Alaribe, A. A. (2011).**The post-treatment effects of enterobiasis on the occurrence of enuresis among children in Calabar , Nigeria. Asian Pacific Journal of Tropical Medicine. 315-319.

Paknazhad, N.; Mowlavi, G.; Dupouy Camet, J.; Jelodar, M. E.; Mobedi, I.,; Makki, M. and Najafi, F. (2016). Paleoparasitological evidence of pinworm (*Enterobius vermicularis)* infection in a female.

Pang , Y. ; Zhao, Y.; Li, S. ; Zhao , Y.; Li , J.; Hu, Z. ; Zhang , C.; Xiao, D. and Yu, A. (2019) . Engineering the oleaginous yeast *Yarrowia lipolytica* to produce limonene from waste cooking oil. Biotechnol Biofuels .Vol.12(241)p:1-18.

Paniker, C. K. J. and Ghosh, S. (2013). Paniker's textbook of medical parasitology.7ed. Jaypee brothers medical puplisher, India.190-194.

Parthiban ,R.; Vijayakumar, S.; Prabhu, S. and Yabesh , J. (2015). Quantitative traditional knowledge of medicinal plants used to treat livestock diseases from Kudavasal taluk of Thiruvarur district, Tamil Nadu, India. Brazilian journal of pharmacognosy. 26 ,p:109–121.

Patsantara, G. G.; Piperaki, E. T.; Bakoula, C. T. and Kanariou, M. G. (2016). Immune responses in children infected with the pinworm *Enterobius vermicularis* in central Greece. Journal of Helminthology.90: 337–341.

Paw, M.; Begum, T.; Gogoi, R.; Pandey, S. K. and Lal, M. (2020). Chemical Composition of *Citrus limon* L. Burmf Peel Essential Oil from North East India , Journal of Essential Oil Bearing Plants.:23(3):1-8.

Pehlivanoğlu, B.; Aydın Türk, B.; İşler, S.; Özdaş, S. and Abeş,M. (2019). Findings in Appendectomies with *Enterobius vermicularis* Infection: Pinworm Is Not A Cause of Appendicitis. Turkiye Parazitolojii Dergisi, 43.(1): 21–25.

Phukan, U. J.; Jeena, G. S.; Tripathi, V. and Shukla, R.(2017). Regulation of Apetala2/Ethylene Response Factors in Plants ,Journal of Front. Plant Sci.Vol.8(150.(

Piperaki , E.T.; Spankos , G. ; Patsantara, G. ; Vassalou, E. ; Vakalis , N. and Tsakris , A . (2011) . Characterization of *Enterobius vermicularis* in a human population, employing a molecular-based method from adhesive tape samples. Mol. Cell Probes; 25(2-3): 121-125.

Prakash, V.(2018). Terpenoids as cytotoxic compounds: A perspective. Phcog. Wolters Kluwer – Medknow.Vol .12 (24) :166-76.

Presterl, E.; El-Schahwi, M. D.; Lusignani, L. S.; Paula, H. and Reilly, S. (2019). Parasites and Parasitic diseases. Springer Nature Switzerland. 161-163.

Rahi, A. A. and Morad, E. E. (2017). Diagnosis of *Enterobius vermicularis* by scotch tape from various areas of Wasit province. World Journal of Pharmaceutical and Life Sciences WJPLS, 3.(7): 49-51.

Rao, K. N. and Venkatachalam, S. R. (2000). Inhibition of dihydrofolate reductase and cell growth activity by the phenanthroindolizidine alkaloids pergularinine and tylophorinidine: the in vitro cytotoxicity of these plant alkaloids and their potential as antimicrobial and anticancer agents. Toxicol. Vitro 14, 53–59 .

Rassem ,H. H. A.; . Nour, A. H . and Yunus , R. M. (2016). Techniques For Extraction of Essential Oils From Plants: A Review Australian Journal of Basic and Applied Sciences, 10(16) , Pages: 117-127.

Ravichandran, C.; Badgujar, P.C.; Gundev, P. and Upadhyay, A. (2018). Review of toxicological assessment of d-limonene, a food and cosmetic additive. Food Chem. Toxicol., 12, 668–680 .

Reinhard, K. J.; Araújo, A. and Morrow, J. J. (2016). Temporal and spatial distribution of *Enterobius vermicularis* (Nematoda: Oxyuridae) in the prehistoric Americas. Korean Journal of Parasitology, 54. (5): 591–603.

Reis-Giada, M.L. (2014). In Oxidative Stress and Chronic Degenerative Diseases – A Role for Antioxidants,J.A. Morales-Gonzalez (Ed), Intech. Open Limited, London,United Kingdom, p: 87–112 .

Rhadi, H. A; Zahra, A. A. and Abdul-Jabar, S. (2018). Prevalence of intestinal parasites in Basrah city, Iraq. International Jornal of science and reserch (JJSR), 8. (2): 174-180.

Riaz, Z. ; Arslan, M. ; Kiani, A. K. and Azhar, S. (2014). CoSMoS: A BIM and wireless sensor based integrated solution for worker safety in confined spaces.Automation in Construction, 45,p :96–106.

Ribereaun – Gayon , P. (1972) . Plant phenolics. Oliver and boyd. U. S. A. 254 pp .

Rodrigues, M. S. ; Morelli ,K. A. and Jansen, A. M.(2017). Cytochrome c oxidase subunit 1 gene as a DNA barcode for discriminating Trypanosoma cruzi DTUs and closely related species. Journal of Parasites & Vectors, Vol(10):488,p:1-18.

Rollins, A. A. (2018). Genetic evidence for the prehistoric expansion of *Enterobius vermicularis* parasite and their human hosts in the greater American Southwest. Indiana University.1-184.

Rudko, S. P.; Ruecker, N. J.; Ashbolt, N. J.; Neumann, N. F. and Hanington, P. C. (2017). *Enterobius vermicularis* as a Novel Surrogate for the Presence of Helminth Ova in Tertiary Wastewater Treatment Plants. Applied and Environmental Microbiology, 83.(11): 1–13.

Russo, M.; Bonaccorsi, I.; Costa, R.; Trozzi, A.; Dugo, P. and Mondello, L. (2015). Reduced time HPLC analyses for fast quality control of citrus essential oils. J. Essent. Oil Res. 27,p: 307–315.

Ryan, E. T. (2018). Antiparasitic Agents . In : Long, Sarah S. (eds.)Principles and Practice of Pediatric Infectious Diseases. (Fifth Edition), Elsevier, Inc. 5th.edn. 1587pp.

Sadjjadi , S . M.; Jamshid Rostami ,J. and Azadbakht , M .(2006) . Giardiacidal activity of lemon juice, vinifer and vinegar on *Giardia intestinalis* cysts. Vol. 37 (3).P:24-27.

Saheb, E. J.; Mahdi, S. G.; Mosa, I. S.; Abdul-Karim, M. I. and Khistawi, A. N. (2017). Epidemiology of some parasitic helminthes in Iraq from 2011 until 2015. Iraqi Journal of science, 58. (2): 789-796.

Saki, J.; Khademvatan, S.; Foroutan-Rad, M. and Gharibzadeh, M.(2016). Prevalence of Intestinal Parasitic Infections in Haftkel County,Southwest of Iran International Journal of Infection. Vol.4(4)p: 1-6 .

Saleem, F.; Malik, F. and Fatima, S. (2017). *Enterobius vermicularis* in tubo-ovarian abscess: A rare and interesting incidental finding - A case Report. J. Pak Med Assoc.; 67(4): 630-633 .

Salman, A. O.; Mhaisen, F. T. and Al-Tae, A. R. A. (2019). Intestinal Parasites among Diarrheic Children Attending a Hospital in Al-Rusafa Side, Baghdad City, Iraq. Biological and Applied Environmental Research, 3. (1:(

Samaraei,Q.W.(1983). Distribution of alkaloids and their taxonomic importance In some wild species of the Solanaceae family in Iraq. Master's Thesis, Faculty of Science, University of Baghdad:157p.

Santos, D. M.; Backer, S. and Saintpierre, B. (2020). Single-nucleus RNA-seq and FISH identify coordinated transcriptional activity in mammalian myofibers. Nat Commun 11, 5102.

Santoshi, M. K. and Krishnaiah ,N. (2019). A clinical Study on atherapeutic Effect of *Katu Taila Pichu* in *Pureeshaja Krimi* W.S.R to *Enterobius Vermicularis* Infestation . International Journal of Ayurvedic Medicine 10 (1):82-88 .

Saravi, K. H. ; Fakhar, M.; Nematian, J. and Ghasemi, M. (2016). Co-infection with *Enterobius vermicularis* and *Taenia saginata* mimicking acute appendicitis. Journal of Infection and Public Health, 9. (4): 519–522.

Satoskar, A. R.; Simon, G. L.; Hotez, P. J. and Tsuji, M. (2009). Medical parasitology. Landes Biosience, USA.P: 1-7.

Sawatdee, S. ; Atipairin, A. ; Yoon ,A. S. ; Srichana, T.; Changsan, N. and Suwandecha, T .(2019). Formulation Development of Albendazole-Loaded Self-Microemulsifying Chewable Tablets to Enhance Dissolution and Bioavailability. Pharmaceutics journal, Vol.11(134).

Schroeder, J. C.; Jones, D. and Maranich, A. (2019). Peripheral Eosinophilia Found in Pediatric *Enterobius vermicularis* Infections. Clinical Pediatrics, 58. (1),p: 13–16.

Shah, B. B. and Mehta, A. A. (2018). In vitro evaluation of antioxidant activity of d–limonene. Asian Journal of Pharmacy and Pharmacolog,4(6):883-887.

Shakir, I. K. and Salih, S. J.(2015). Extraction of Essential Oils from *Citrus* By-Products Using Microwave Steam Distillation. Iraqi Journal of Chemical and Petroleum Engineering,Vol.16(.3):11- 22 .

Sharma, M.; Kaul, R, and Chander, B. (2018). *Enterobius vermicularis* infestation leading to meckel's diverticulitis in an adolescent boy: An extremely rare presentation. Journal of Laboratory Physicians, 10. (1): 106-108.

Sherwani, S. K.; Khan, M. M.; Khan, M. U.; Shah, M. A. and Kazmi, S. U.(2013). Evaluation of In Vitro anthelmintic activity of *Cymbopogon citratus* (lemon grass) extract. Int. J. of Pharm. & Life Sci. , Vol. 4 (6),p: 2722-2726 .

Shija, K. M. R.; Nondo, S. O.; Mloka, D.; Sangeda, R. Z. and Bwire,G. M. (2020). Effects of lemon decoction on malaria parasite clearance and selected hematological parameters in *Plasmodium berghei* ANKA infected mice. BMC Complementary Medicine and Therapies,V.20(24).p:1-12

Sindle, A . and Martin, K.(2020). Art of Prevention: Essential Oils - Natural Products Not Necessarily Safe. International Journal of Women's Dermatology. 7 ,p: 304–308.

Smythe ,A. B.; Holovachov, O. and Kocot ,K. M.(2019). Improved phylogenomic sampling of freeliving nematodes enhances resolution of higher-level nematode phylogeny 19:121p:1-15.

Sorour, S. S. G.; El-Hawary, N. M.; El-Morsey, A.; Elmajdoub, L. O.; Omar, M. and Sultan, K.(2020). Molecular and phylogenetic study based on two mitochondrial DNA genes of rabbit pinworm "Passalurus ambiguus". Asian J. Anim. Vet. Adv., 15: 20-24.

Soulimani, R.; Bouayed, J. and Rakesh, K. J.(2019). Limonene: Natural monoterpene volatile compounds of potential therapeutic interest American Journal of Essential Oils and Natural Products 2019; 7(4): 01-10.

Sravanthi, M.; Swetha, G.; Maheshwari, A.; Shravya, K.; Nureen, B. H. and Raju, K.(2020). *In-vitro* anthelmintic activity of *Citrus limon* leaf and its phytochemical investigation .Journal of Pharmacognosy and Phytochemistry.9(3): 816-818.

Stephane, F. F. Y. and Jules ,B. K. J.(2020). Terpenoids as Important bioactive constituents of essential oils extraction of bioactive compounds from medicinal plants and herbs. IntechOpen.p: 1371 .

Sumanto, D. ; Sayono, S. and Mudawamah, P.L.(2021). *Enterobius vermicularis* larvae in urine sample of female student: The first case report in Indonesia. Journal of Microbiol Exp.;9(1):1–2 .

Sun, J. (2007). D- Limonene: Safety and clinical applications. Altern. Med. rev. Inc. Vol.12(3).p:259-264.

Taha, H. A.; Soliman, M. I. and Banjar, S. A. N. (2013). Intestinal parasitic infections among expatriate workers in Al-Madina Al- Munawarah, Kingdom of Saudi Arabia. Tropical Biomedicine, 30. (1): 78–88.

Taher, J. H. (2017). Medical Parasitology. Ministry of Higher Education and Scientific research. Al-Furat Al-Awsat Technical University.103-105.

Tanaka, Y.; Tsuda, M.; Yasumoto, K.; Terachi, T. and Yamagishi, H. (2014). The complete mitochondrial genome sequence of *Brassica oleracea* and analysis of coexisting mitotypes. Curr Genet 60:277–284.

Tavan, A. ;Mikaeili, F.; Sadjjadi ,S.M. ;Bajelan, S. ;Mahmoudv, H. and Sharifdini, M. (2021). Prevalence and genotype distribution of *Enterobius vermicularis* among kindergarteners in Shiraz and Khorramabad1 cities, Iran. Asian Pac. J. Trop. Med.; 13(7): 308-313.

Tayfur, M. and Balci, M. (2019). Parasites : A Retrospective Study of 2400 Cases of Acute Appendicitis. Nigerian Journal of Clinical Practice, 22. (2): 270–275.

Taylor, A.; Saichua, P.; Rhongbutsri, P.; Tiengtip, R.; Kitvatanachai, S. and Taylor, W. R. J. (2018). A preliminary epidemiological study of pinworm infection in Thaklong Municipal Early Childhood Development Center and Rangsit Babies' Home, Pathum Thani, Thailand. BMC Research Notes, 11. (1): 1–6.

Temsah, K. A. ; Fattah, D. A.; El Kholy , A. A. and Elsamanoudy , M. I.(2021) . Efficacy of Albendazole Mass Treatment Alone Compared to Combined Albendazole – Flubendazole Regimen for Treatment of Resistant *Enterobius vermicularis* Infection in Children *Enterobius* .The Egyptian Journal of Hospital Medicine ,Vol. 84, Page 2071-2077 .

Thawabteh, A.; Juma, S.; Bader, M.; Karaman, D.; Scrano, L.; Bufo, S.A. and Karaman, R. (2019). The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. Toxins, 11, 656.

Tomanakan, K.; Sanpool, O.; Chamavit, P.; Lulitanond, V.; Intapan, P. M. and Maleewong, W. (2018). Genetic variation of *Enterobius vermicularis* among schoolchildren in Thailand. Journal of Helminthology. 94: 1–5.

Trivedi, S.; Aloufi, A.A.; Ansari, A.A. and Ghosh, S.K. (2016). Role of DNA barcoding in marine biodiversity assessment and conservation: an update. Saudi, J. Biol. Sci.; 23:161–71.

Tsai, C. –Y.; Junod, R.; Jacot-Guillarmod, B.; Charles; Z. S. and Bongiovanni, M.(2017). Vaginal *Enterobius Vermicularis* Diagnosed on Liquid-Based Cytology During Papanicolaou Test Cervical Cancer Screening: a Report of Two Cases and a Review of the Literature. Journal of Diagnostic Cytopathology , 17 (236) p:1-18.

Upadhyaya ,S.(2018).*Citrus limon* L Burmf peel: Potential anthelmintic agent against Indian Earthworm *Eicinia foetida*. Journal of Drug Delivery & Therapeutics. 2018; 8(5):248-250.

Upadhyaya, S.; Khatiwora , E. and Bora, D.K.(2019). Estimation of total Phenol and flavonoid contents of *Citrus limon* L Burmf leaves from North Eastern region of India. Journal of Drug Delivery & Therapeutics .Vol. 9(2):40-42.

Velderrain-Rodríguez, G.R.; Palafox-Carlos, H. ; Wall-Medrano, A. ; Ayala-Zavala, J.F, ;Chen C.Y.; Robles-Sánchez, M. ;Astiazaran-García, H. ;Alvrez-Parrilla, E and González-Aguilar, G.A.(2014) . Phenolic compounds : their journey after intake. Food Funct.;5(2):189-97 .

Verrest, L. and Dorlo, T.P.C. (2017). Lack of clinical pharmacokinetic studies to optimize the treatment of neglected tropical diseases: a systematic review. Clin. Pharmacokinet;56:583-606.

Vieira, M. S.; Hiltensperger, M.; Kumar, V.;Zegarra-Ruiz, D.;Dehner, C.; Khan, N. ;Costa, F.R.C. ; Tiniakou, E.; Greiling, T.; Ruff, W. ; Barbieri, A. ; Kriegel, C.; Mehta, S.S. ;Knight, J.R. ; Jain, D. ; Goodman, A.L. and Kriegel, M.A.(2018).

Translocation of a gut pathobiont drives autoimmunity in mice and humans. Sci. Mar. 9;359(6380):1156-1161.

Walochnik, J . and Duchêne, M. (2016). Molecular Parasitology: Protozoan Parasites and their Molecules. Springer,p:547 .

Wang, S.; Yao, Z.; Hou, Y.; Wang, D.; Zhang, H.; Ma, J. and Liu, S. (2016) . Prevalence of *Enterobius vermicularis* among preschool children in 2003 and 2013 in Xinxiang city, Henan province, Central China . Parasite, 23. (30): 1-5.

Wang, X. –F. ; Hu, Y.; Li , J. –H. ; Zhang, L. ; Gong , X. and Huang , H .(2020). Analysis of the basic components and free amino acid composition of pineapple fruit vinegar. E3S Web of Conferences 185, 04047 .ICEEB.J. p:1-3.

Weerakoon ,K. G. and McManus, D. P.(2017). Cell-Free DNA as a Diagnostic Tool for Human Parasitic Infections.Vol.32(5)p:378-391.

Wendt, S.; Trawinski, H.; Schubert, S.; Rodloff, A. C.; Mössner,J. and Lübbert, C. (2019). Diagnostik und Therapie des Madenwurmbefalls. Deutsches Arzteblatt International, 116. (13): 213– 219.

Wink, M.(2012). Medicinal plants: a source of anti-parasitic secondary metabolites. Journal of Molecules. 31;17(11):12771-91 .

Wu , P.-S.; Kuo, Y.-T.; Chen, S.-M. ; Li2, Y. and Lou, B.-S. (2014) . Gas Chromatography-Mass Spectrometry Analysis of Photosensitive . Chromatogr. Sep. Tech. J .6(10).

Xia, T.; Zhang, B.; Duan, W. ; Zhang , J. and Wang, M. (2020) . Nutrients and bioactive components from vinegar: A fermented and functional food .Journal of Functional Foods, 64.p:1-14.

Xing, F. X.; Yea, H.; Yanga, J.; Chana, J. F. –W.; Setoa, W. –K. ; Paia P. M. –C.; Yuena, K. -Y. and Hungd, D. L. -L. (2018). Fatal pancytopenia due to albendazole treatment for strongyloidiasis. I.D.Cases.Vol.12 ,p:112–116.

Xinmiao, L.; Zhao , S.; Ning , Z.; Zeng , H.; Shu , Y.; Tao , Xiao2 O. C. ; Lu ,C. and Liu ,Y. (2015). *Citrus* fruits as a treasure trove of active natural metabolites that potentially provide benefits for human health. Chemistry Central Journal (2015). 9:(68).p:1-14.

Yadava, N. ; Yadava, R. and Goyalb, A. (2014). Chemistry of Terpenoids. Int. J. Pharm. Sci. Rev. Res., 27(2), P: 272-278.

Yamuna, P. ; Abirami, P.; Vijayashalini,P and Sharmila, M.(2017).GC-MS analysis of bioactive compounds in the entire plant parts of ethanolic extract of *Gomphrena decumbens* Jacq. Journal of Medicinal Plants Studies, Vol. 5(3), p: 31-37.

Yang, C.; Li, Z. ; Shi, Z. ; He, K.; Tian, A.; Wu, J. ; Zhang, Y. and Li, Z. (2014) . Regulation of cell survival by the HIP-55 signaling network. Mol Biosyst 10(6):1393-1399.

Yang, J.; Tauschek, M.; and Robins-Browne, R. M. (2011). Control of bacterial virulence by AraC-like regulators that respond to chemical signals. Trends Microbiol. 19, 128–135 .

Yazgan, P.; Utku, D. E. and Sirkeci,I.(2015). Editorial: Syrian crisis and migration. Migration Letters, Volume: 12, No: 3, pp. 181 – 192.

Zandalinas, S.I.; Sales, C.; Beltrán, J. ;Gómez-Cadena,s A. and Arbona, V. (2017). Activation of Secondary Metabolism in *Citrus* Plants Is Associated to Sensitivity to Combined Drought and High Temperatures. Front. Plant Sci.J. V.7 , Article1954.

Zeibig,E.A.G.–B.(2013). Clinical Parasitology, Apractical approach .2ed.Elsevier, Saunders press,p.385.

Zelck, U.E.; Bialek, R. and Weiss, M. (2011). Molecular phylogenetic analysis of *Enterobius vermicularis* and development of an 18S ribosomal DNA-targeted diagnostic PCR. Journal of Clinical Microbiology 49, 1602–1604.

Zeng, Y.; Ye, W.; Kerns, J.; Tredway, L.; Martin, S.; and Martin, M. (2015). Molecular characterization and phylogenetic relationships of plant-parasitic nematodes associated with turfgrasses in North Carolina and South Carolina, United States. Plant Dis. 99:982-993.

Zenner , L.;Callait , M.P.; Granier , C. and Chauve , C.(2003). *In vitro* effect of essential oils from *Cinnamomum aromaticum* , *Citrus limon* and *Allium sativum* on two intestinal flagellates of poultry, *Tetratrichomonas gallinarum* and *Histomonas meleagridis* .Parasite, 10, 153-157

Zhang, B. ; Xia, T.; Duan, W. H. ; Zhang, Z. J.; Li, Y.; Fang, B. and Wang, M. (2019). Effects of organic acids, amino acids and phenolic compounds on antioxidant characteristic of Zhenjiang aromatic vinegar. Molecules, 24, 3799.

Zhang, Y.; Xu, W.W. ; Guo, D.H.; Liu , Z.X. and Duan, H.(2015). The complete mitochondrial genome of *Oxyuris equi*: Comparison with other closely related species and phylogenetic implications. Exp. Parasitol., 159: 215-221.

Zouari, M. ; Louati, H.; Abid, I.; Trabelsi, F. ; Ben Dhaou, M.; Jallouli, M.and Mhiri, R.(2018).*Enterobius vermicularis*: A Cause of Abdominal Pain Mimicking Acute Appendicitis in Children. ARetrospective Cohort Study .Arch Iran Med journal. 21(2):67-72.

Zouari, M. and Mhiri, R. (2019). Reply to "*Enterobius vermicularis*: Uncommon Clinical Presentations" . Arch Iran Med, 22 .(2):106.

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Abbreviations List

Abbreviations	Details
amu	Atomic mass unit
Ang	Angstrom
BLAST	Basic Local Alignment Search tool
c^o	Celsius degree
*COX*1	Cytochrome oxidase subunit 1
DNA	Deoxyribonucleic acid
FTIR	Fourier Transform Infrared (FTIR) spectroscopy
g	Gram
IgE	Immunoglobulin type E
L	Liter
mA	Milliampere
mg	Milligram
ml	Milliliter
µl	Microliter
NCBI	National center for biotechnology information
nm	Nanometer
PCR	Polymerase chain reaction
pH	Potential of hydrogen
rpm	Revolution per minute
ST1	Spikes tactical 1buffer
ST3	Spikes tactical 1buffer
TBE	Tris Borate Ethylenediaminetetraaceticacid (EDTA)