

Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Babylon
College of Sciences for Women
Department of Biology



Effect of Secondary Plant Metabolites Extracted from Some Medicinal Plants Against *Candida* species Isolated from Clinical Samples in Babylon Province

A Thesis

**Submitted to the Council of the College of Sciences for Women/
University of Babylon in Partial Fulfillment of the Requirements for
the Degree of Master in Biology**

By

Zainab Mohammed Karim Al Qureshi

Supervised By

Prof. Dr.

Hussein Jebur Hussein

Prof. Dr.

Abeer Fauzi Al-Rubaye

2022AD

1444 AH

بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِیْمِ

(قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا لَّكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ تَنفَدَ

كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا)

صدق الله العلي العظيم

سورة الكهف

الاية (109)

Supervisors Certification

We certify that this thesis entitle (**Effect of Secondary Plant Metabolites Extracted from Some Medicinal Plants Against *Candida* species Isolated from Clinical Samples in Babylon Province**) was prepared under our supervisionat the department of Biology, College of Science for Women, University of Babylon as a partial requirement for the degree of Master in Biology.

Signature:

Prof. Dr. Hussein Jaber Hussein

College of Science for women

University of Babylon

Date: / / 2022

Signature:

Prof. Dr. Abeer Fauzi Al-Rubaye

College of Science for women

University of Babylon

Date: / / 2022

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

Signature:

Prof. Dr. Hadi Meziel Kudhair

Head of Microbiology Department

College of Science for Women

University of Babylon

Date: / / 2022

Certification of Examining Committee

We, the member of examining committee, certify that we have read this thesis entitled (**Effect of Secondary Plant Metabolites Extracted from Some Medicinal Plants Against *Candida* species Isolated from Clinical Samples in Babylon Province**) and after examining the Master student (**Zainab Mohammed Karim**) in its contents in/ / / 2022 and that in our opinion it is adequate as a thesis for the degree of Master in Biology with degree ().

Committee Chairman:

Signature:

Name: Dr. Huda Jassim Mohammed

Scientific order: Professor

Address: Collage Science of for women\ University of Babylon

Date:

Committee Member

Signature:

Name: Dr. Ivan Ibrahim Merhej

Scientific order: Professor

Address: Science of Collage \ University of Babylon

Date:

Committee Member

Signature:

Name: Dr. Entesar Abd alhamid Mahmoud

Scientific order: Lecture Dr.

Address: AlQasim University

Date:

Committee Member (Supervisor)

Signature:

Name: Hussein Jabr Hussein

Scientific order: Professor

Address: Collage of Science for women\ University of Babylon

Date:

Committee Member (Supervisor)

Signature:

Name: Abeer Fauzi Murad

Scientific order: Professor

Address: Collage of Science

for women\ University of Babylon

Date:

Date of examination: / 31 / 10 / 2022

Deanship authentication of Science College for Women Approved for the college committee of graduate studies.

Signature:

Name: Dr. Abeer Fauzi Murad Al-Rubaye

Scientific order: Professor

Address: Dean of Science Collage for women\ University of Babylon

Date

Dedication

To my father, the ideal man
my support, my source of strength, to my mother
thanks to her prayers
to my dear sisters
to my soulmate, my husband.

Zainab

Thanks and appreciation

I thank God - the Almighty - and praise Him, for He is the bestower and favored person before everything. I thank Him for achieving what I aspire to complete my studies. I would like to thank the presidency of the university of Babylon and deanship of the College of Science for Women .

Zainab

Summary

The current study was conducted at University of Babylon, College of Science for women, Department of Biology, The aim of this study was isolation, identification, and controlling of *Candida* species isolated from Vaginal and oral samples by using secondary metabolites compounds such as Alkaloids, Flavonoids, and Terpenoids extracted from three medicinal plants such as flower buds of *Dianthus caryophyllus* L., roots of *Saussurea costus*, and seeds of *Carthamus tinctorius* L. In this study, three types of yeasts were diagnosed *Candida albicans*, *Candida tropicalis*, and *Candida dubliniensis*. Hemolytic and biofilm virulence factors and sensitivity tests were performed by using three types of antibiotics (Fluconazole 150 mg), (Ketoconazole 150 mg) ,(Caspofungin 150 mg). Antifungal activity was achieved in vitro by using agar well diffusion method against *Candida* species by preparing three concentrations for each crude compound (25, 50, and 100) mg/ml and compared with positive control represented by Fluconazole 50mg/ml and negative control represented by 10% dimethyl sulfoxide. The data collected from the study revealed that the crude Terpenoids and Flavonoids compounds extracted from flower buds of *Dianthus caryophyllus* L. showed significant superiority at $P \leq 0.05$ over the fluconazole when applied to *Candida* species especially at 100mg/ml. The data also revealed that, the crude Alkaloids, Flavonoids, and Terpenoids compounds extract from roots of *Saussurea costus* showed reduction at $P \leq 0.05$ in the growth of *Candida* species especially at 100mg/ml compared with negative control. In the same time the crude Alkaloids, Flavonoids, and Terpenoids compounds extract from seeds of *Carthamus tinctorius* L. showed reduction at $P \leq 0.05$ in the growth of *Candida* species especially at 100mg/ml compared with negative control. Finally, it can be concluded that secondary metabolites extracted from flower buds of *Dianthus*

caryophyllus L., roots of *Saussurea costus*, and seeds of *Carthamus tinctorius* L. is most effective in controlling *Candida* species isolated from clinical samples.

Table of Contents

| Sequence | Contain Chapter One : Introduction | Page |
|---|--|------|
| 1.1. | Introduction | 1 |
| 1.2 | Aim of the study | 3 |
| Chapter Two-2- Literature Review | | |
| 2.1 | Medicinal plant | 4 |
| 2.2 | History of the use of medicinal herbs | 6 |
| 2.3. | <i>(Carnation (Dianthus caryophyllus L).</i> | 7 |
| 2.4 | <i>Saussurea costus</i> | 10 |
| 2.5 | <i>Carthamus tinctorius</i> | 12 |
| 2.6 | Secondary metabolites | 14 |
| 2.6.1 | Alkaloids | 15 |
| 2.6.1.1 | Distribution and localization of alkaloids | 16 |
| 2.6.1.2 | Pharmacological activities of alkaloids | 17 |
| 2.6.2 | Flavonoids | 18 |
| 2.6.2.1 | Distribution of Flavonoids | 18 |

| | | |
|-----------------|--|----|
| 2.6.2.2. | Pharmacological activities of flavonoids | 19 |
| 2.6.3 | Terpenoids | 20 |
| 2.6.3.1 | Distribution of terpenoid | 21 |
| 2.6.3.2 | Pharmacological Activities of Terpenoids | 22 |
| 2.7 | Candida | 23 |
| 2.8. | Candidiasis | 24 |
| 2.8. | Candida species | 24 |
| 2.8.1. | Candida albicans | 26 |
| 2.8.1 | Candida tropicalis | 27 |
| 2.8.3 | Candida dubliniensis | 27 |
| 2.9 | Candidiasis | 27 |
| 2.9.1 | Vaginal Vulvovaginal Candidiasis | 28 |
| 2.9.2 | Orthodontic Candidiasis | 29 |
| 2.10 | Virulence Factor and Resistance | 30 |
| 2.10.1 | Resistance of <i>Candida</i> | 31 |
| 2.10.2. | Fungal Biofilms | 32 |

| | | |
|--|--|----|
| 2.10.3 | Hemolaytic | 34 |
| Chapter Three : Materials and Methods | | |
| 3 | Materials and Methods | 37 |
| 3.1 | Materials | 37 |
| 3.1.1 | Laboratory Instruments | 37 |
| 3.2 | Preparation media | 39 |
| 3.2.1 | Manitol Agar | 39 |
| 3.2.3 | Mcaconky Agar | 30 |
| 3.2.2 | Blood Agar | 40 |
| 3.2.4 | Sabouraud Dextrose Agar | 40 |
| 3.2.5 | Potato Dextrose Agar | 40 |
| 3.2.6 | Chrom Agar Candida | 41 |
| 3.2.7 | Mueller Hinton Agar (MHA) | 42 |
| Chapter Four : Results and Discussion | | |
| 4 | Results and discussion | 47 |
| 4.1 | Distribution of age, gender among studied group | 47 |
| 4.2 | Isolation and identification | 49 |
| 4.2 | Identification by CHROME agar medium | 52 |
| 4.2.2 | Identification by Vitik2 System | 52 |
| 2 | Formation of biofilm | 55 |
| 3.4 | Antifungal activity of three plants (<i>Dianthus caryophyllus</i> <i>Saussurea costus</i> and <i>L. Carthamus tinctorius</i>) against some <i>candida</i> spp. | 57 |
| 4.3.2 | Antifungal activity of <i>Dianthus caryophyllus</i> | |

| | | |
|---|-------------------------------|----|
| | L. against <i>Candida</i> spp | |
| Chapter Five : Conclusions & Recommendations | | |
| 4.3.2 | Conclusion | 76 |
| 5.2 | Recommendations | 76 |
| References | | |
| | References | 77 |

List of Images

| | | |
|------------|---|-----------|
| 4-1 | Colonies color of <i>Candida</i> species on Dextrose agar medium at 30°C for 24-48h ,A. <i>Candida albicans</i> , B. <i>C. tropicalis</i> , C. <i>C. dubliniensis</i> | 50 |
| 4-4 | Colonies color of <i>Candida</i> species on CHROM agar medium at 30°C for 24-48h . ,A. <i>C. albicans</i> ,B. <i>C. dubliniensis</i> , c. <i>C. tropicalis</i>). | 53 |
| 4-5 | Hemolysin production of <i>Candida</i> spp species blood agar medium on 37°C for 48 h, a. <i>Candida albicans</i> ,b. <i>C. tropicalis</i> , c. <i>C. dubliniensis</i>). | 53 |
| 4-6 | hemolysis by <i>Candida</i> species in vaginal infections | 55 |
| 4-8 | A. Showing some biofilm readings,B. howing the highest biofilm reading and the least reading, C. hows biofilm formation on the walls of the tube | 56 |
| 4-11 | Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml of <i>Dianthus caryophyllus</i> L. flower buds against <i>Candida</i> species | 60 |
| 4-13 | Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml against of <i>Saussurea costus</i> roots <i>Candida</i> species | 66 |
| 4-15 | Anti-candidiasis activity of the crude Terpenoid compounds extracted from (<i>Carthamus tinctorius</i> L.) seeds | 72 |

List of Tables

| No | Topic | Page Number |
|-----|---|-------------|
| 3-1 | List of Instruments and Equipment used in this study | 37 |
| 3-2 | Chemical and biological Materials used in this study | 38 |
| 3-3 | Culture media used in the Laboratory | 39 |
| 4-1 | History of patient with vaginal infection | 48 |
| 4-2 | History of patient with orthodontic infection | 49 |
| 4-3 | The frequency of type of infections according to the specimen's type | 51 |
| 4-4 | The frequency of type bacteria and yeast according to the specimen's type | 51 |
| 4-5 | Distribution candida spp infections according of specimen's type | 51 |
| 4-6 | frequency of candida <i>spp</i> in Vaginal and Orthodontic infections | 54 |
| 4-7 | Anti-candidiasis activity of the crude Terpenoid compounds extracted from <i>Dianthus caryophyllus</i> L. flower buds | 58 |
| 4-8 | Anti-candidiasis activity of the crude Flavonoid compounds extracted from <i>Dianthus caryophyllus</i> L. flower buds | 58 |
| 4-9 | Anti-candidiasis activity of the crude Alkaloid compounds extracted from | 64 |

| | | |
|------|---|----|
| | <i>Saussurea costus</i> roots | |
| 4-10 | Anti-candidiasis activity of the crude Flavonoid compounds extracted from <i>Saussurea costus</i> roots | 65 |
| 4-11 | Anti-candidiasis activity of the crude Terpenoid compounds extracted from <i>Saussurea costus</i> roots | 71 |
| 4-12 | Anti-candidiasis activity of the crude Alkaloid compounds extracted from (<i>Carthamus tinctorius</i> L) seeds | 71 |
| 4-13 | Anti-candidiasis activity of the crude Flavonoid compounds extracted from (<i>Carthamus tinctorius</i> L.) seeds | 71 |
| 4-14 | Anti-candidiasis activity of the crude Terpenoid compounds extracted from (<i>Carthamus tinctorius</i> L.) seeds | 72 |

List of Figures

| No | Topic | Page Number |
|------|---|-------------|
| 4-2 | The distribution of infectious isolates in vaginal infections | 51 |
| 4-3 | The distribution of infectious isolates in Orthodontic infections | 52 |
| 4-9 | Biofilm formation by <i>Candida</i> species in vaginal infections | 56 |
| 4-10 | Biofilm formation by <i>Candida</i> species in orthopedic infections | 57 |
| 4-11 | Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml against <i>Candida</i> species, | 61 |

List of Abbreviations

| Term | Meaning |
|---------------|-------------------------------------|
| BV | bacterial vaginosis |
| CNS | central nervous system |
| DMAPP | dimethylallyl diphosphate |
| D.W | Distiller water |
| EPC | esophageal candidiasis |
| ICUs | intensive care units |
| IPP | isopentenyl diphosphate |
| MVA | mevalonic acid |
| MHA | Mueller Hinton agar |
| GlcNAc | N-acetylglucosamine |
| NGS | Next-generation sequencing |
| NACs | non-albicans <i>Candida</i> species |
| OPC | oropharyngeal candidiasis |
| SMs | secondary metabolites |
| TV | <i>Trichomonas vaginalis</i> |
| VVC | Vulvovaginal candidiasis |
| DMSO | Dimethyl sulfoxide |
| RVVC | recurrent vulvovaginal candidiasis |
| ROS | reactive oxygen species |

Chapter One

Introduction

1.1 Introduction :

With the increasing incidence and complexity of diseases threatening human health and Increasing resistance to various types of antibiotics the need for novel and effective bio-molecules is of paramount importance, which brings forward natural products/plants as the pipeline of tomorrow for drug (Anand *et al.*,2019).

It is considered potential pharmacies grown in the wild and have been co-existed and co-evolved alongside human civilizations since the beginning of life on Earth. Since ancient times, human life has been revolving around plants as they were used for their curative nature to alleviate human pain and have been the focal point of many researchers since the dawn of medicine. For centuries, medicinal plants have been used as remedies for human ailments and diseases because they contain components of therapeutic value. (Ernst *et al.*, 2016).

Candidal vulvovaginitis is common it is responsible for a third of all cases of vulvovaginitis in reproductive-aged women, and 70% of women report having had candidal vulvovaginitis at some point in their lifetimes. About 8% of women suffer recurrent candidal vulvovaginitis the most common responsible pathogen (Buggio *et al.*,2019).

Candida albicans (in about 90% of cases), with most of the remaining cases caused by *Candida glabrata*. It is important to recognize that detailed epidemiological data is not available for this disease process because of the wide availability of over-the-counter treatments, many patients with candidal vulvovaginitis likely do not present for care (Aguirre *et al.*,2019).

Removable orthodontic appliances and full dentures provide an excellent environment for adhesion and colonization of pathogenic and

non-pathogenic organisms that can cause infectious ulcers in the oral mucosa (Simmons *et al.*,2014). Yeasts are not inherently pathogenic, which means that very few of them can cause diseases in a healthy person, and immunodeficiency is a necessity for their pathogenicity (Atarod *et al.*,2020).

Various types of *Candida* are known as opportunistic pathogens, which are located in the oral cavity common types that lead to diseases are *Candida albicans*, and *Candida dubliniensis*, which could replicate in cases of diminished host defense and become pathogenic despite their limited virulence (Heidari *et al.*,2019)

Candida's adhesion to the host's mucous membranes, acrylic surfaces of removable orthodontic appliances, and denture prostheses lead to the production of proteolysis enzymes that damages mucosal cells resulting in denture stomatitis (Alhasani *et al.*.,2020).

1.2 Aim of study :

Biological Control of *Candidia* species isolated from clinical samples (vaginal and orthodontic) metabolites extracted from three medicinal plants (*Dianthus caryophyllus L*, *Saussurea costus* , *Carthamus tinctorius*) and compared with antibiotic .

Chapter Two

Literature Review

Literature Review :

2.1 Medicinal plant:

Nature is always a golden sign to show the prominent phenomena of coexistence. Natural products from plants, animals and minerals are the basis for treating human diseases (Firenzuoli and Gori, 2007). Medicinal plants are presently in demand and their acceptance is increasing progressively undoubtedly, plants play an important role by providing essential services in ecosystems. Without plants, humans and other living organisms cannot live in a way living should be. Anyway, herbals especially medicinal herbs have constantly acted as an overall indicator of ecosystem health (Singh, 2002).

Medicinal plants have undoubtedly been considered by human beings since ancient times. It can be said that before the history and since the early humans recognized and exploited the plants around them for use as fuel, clothing, shelter and food, they became aware of their properties more or less medicinal plants have been transformed into one of the oldest sciences in countries such as China, Greece, Egypt and India. In ancient Persia, plants were commonly used as a drug and disinfectant and aromatic agent the who, through participation in additionally has led efforts to develop a harmonized international pharmacopoeia (Hamilton, 2004 and WHO, 2018).

In fact, the use of medicinal plants for the treatment of diseases dates back to the history of human life, that is, since human beings have sought a tool in their environment to recover from a disease, the use of plants was their only choice of treatment (Ugbogu and Akukwe, 2009).

Chapter Two Literature Review

More than tenth of the plant species (over 50 000 species) are used in pharmaceutical and cosmetic products. However, the distribution of medicinal plants across the world is not uniform (Rafieian-Kopaei, 2012).

Medicinal herbs are mainly collected from the wildlife population. Indeed, the demand for wildlife sources has increased by 8%-15% per year in Europe, North America and Asia in recent decades and before its independence, attempts have been made to document the medicinal flora of Mauritius. Indeed, the first documentation by the botanist Bouton to record medicinal plants of Mauritius was published in 1856 (Verma and Singh, 2008). (The Royal Society of Arts and Sciences of Mauritius, 2018).

The term medicinal plant refers to a variety of plants that have medicinal properties. These plants are a rich source of compounds that can be used to develop drug synthesis (Abdul, 2012).

Medicinal plants are used for treatment because they have certain properties, including synergistic actions the constituents of the plant may interact with each other, and this interaction can be beneficial for both or adverse to either of them or eliminate the harmful effects of both. Plant-derived compounds can dramatically improve hard-to-treat illnesses, such as cancer plant components are also characterized by their ability to prevent the development of certain diseases the toxicity and adverse effects of conventional and allopathic (Abdul, 2012).

Medicines have also been important factors in the sudden increase in population demands and increase in the number of herbal drug manufactures as well as a reduction in the use of chemical drugs therefore, there is an increasing demand to develop new antimicrobial agents that are able to decrease the use of antibiotics and to face

resistance development. This has directed researchers to isolate and identify new bioactive chemicals from plants to act against microbial resistance (Chavan *et al.*,2018) .

2.2 History of the Use of Medicinal Herbs:

There are recipes for folk or traditional healing practices that use the biological activity of various substances that are derived from plants to treat different diseases, including those that are caused by bacteria. Many of these traditional treatments are still widely used today. Moreover, some commercially established drugs used in modern medicine had an initial crude form in folk medicine (Kong *et al.*, 2020).

Given the fact that at that time there was insufficient information about the causes of the disease, useful plants for treating them, and the ways of using them for such purpose, everything was empirical over time, the reasons for the use of certain medicinal plants for treatment of certain diseases were discovered consequently the use of medicinal plants gradually rejected the empirical framework and was limited to the facts. The earliest written evidence of the use of medicinal plants for preparation of drugs has been found on a Sumerian clay slab from Nagpur dating back to nearly 5000 years ago therefore, great attention has been paid to the secondary metabolites secreted by plants in tropical regions that may be developed as medicines (Qiu,2007 and Guerrier *et al.*,2018) .

2.3. *Carnation (Dianthus caryophyllus L).*

Belong is to *Caryophyllaceae* family the probable origin of the plant is mediterranean region (Kamil *et al.*, 2020). The plant has many synonyms such as *Dianthus acinifolius* Schur, *Dianthus arbuscula* Lindl., *Dianthus arrectus* Dumort., *Dianthus binatus* Schur , *Dianthus caryophyllus* var. *coronarius* L. , *Dianthus coronarius* (L.) Burm.f., *Dianthus corsicus* Link

Chapter Two Literature Review

ex Spreng., *Dianthus kayserianus* Schur, *Dianthus longicaulis* Costa, *Dianthus miniatus* A.Huet ex Nyman, *Dianthus moschatus* J.F.Gmel., *Dianthus multinervis* Vis. and *Tunica morrisii* (Hance) Walp (Lim , 2012).

The plant is annual or perennial, 15-60 cm long, branched, glabrous herb. Leaves are linear lanceolate, apex acute, margin smooth or ciliate at base flowers are solitary or in clusters at tips of branches. Epicalyx scales are 4-6 in number, broad-ovate, abruptly mucronate at apex, herbaceous, appressed to calyx, covering one fifth to one quarter the length of calyx tube. Calyx tube is cylindrical, 20-30 mm long petals are 5, limb exerted, triangular obovate, toothed at apex, auricle absent, pink-red or white, sometimes spotted with darker centers, claw cuneate, glabrous and the optimum growing temperature for carnation *Dianthus caryophyllus* L. is 18–24°C, and they prefer relatively cooler climates (Chandra *et al.*, 2016 and Higashiura *et al.*, 2020).

Taxonomic classification according to (Komarov *et al.*,1970).

Kingdom: Plantae

Subkingdom: Viridiplantae

Infrakingdom: Streptophyta

Superdivision: Embryophyta

Division: Tracheophyta

Subdivision: Spermatophytina

Class: Magnoliopsida

Superorder: *Caryophyllanae*

Order: *Caryophyllales*

Family: *Caryophyllaceae*

Genus: *Dianthus L*

Species: *Dianthus caryophyllus*

Dianthus caryophyllus is a common name carnation that is a member of the Caryophyllaceae family, developed about 2,000 years ago, raised in many areas of the world and assumed to be a Mediterranean native carnation is one of the foremost common commercial flowers, ranking beside Roses in grandeur. *D. caryophyllus* is a perennial herbaceous plant with a length of up to 80 cm. The leaves are greyish to blue-green, soft, up to 15 cm in length (Madhuri and Barad, 2018).

The stems are woody at the root, with herbaceous branches. *D. caryophyllus* is a medicinal value. These have largely become used in the treatment of stomach aches and teeth, in the treatment of wounds such as cardiovascular, cardiac, alexi- and vermifugal diseases. Traditionally, medicines are used to cure injury in China, Japan and Korea, gastrointestinal conditions, and various other diseases (Chandra *et al.*, 2016).

Dianthus caryophyllus contains a variety of secondary metabolites such as alkaloids, triterpenes, coumarins, phenolic acids, anthocyanins, essential oil, pelargonidin, isosalipurposide, volatile oil and other chemical compounds; that benefit Pharmaceuticals in various diseases such as anti-cancer, anti-viral, anti-microbial, antifungal, insecticide, repulsive, antioxidant, rheumatoid, and analgesic effects (Al-Snafi, 2017).

2.4. *Saussurea costus*

Saussurea costus (Falc.) Lipschitz, a perennial herbaceous plant, one of the best-known species within this genus, is commonly known as costus in English. It has several synonyms, such as *Aucklandia costus* Falc., *Aplotaxis lappa* Decne., *Saussurea costus* (Falc.) Sch.-Bip., *Aucklandia lappa* Decne, *Saussurea lappa* (Decne.) C. B. Clarke., *Theodorea costus* O. Kuntz (Houchi and Messasma, 2022).

Several workers have reported on the different biological activities of *Saussurea costus* in various in vitro and in vivo test models. Pharmaceutical effects investigations of extracts from *Saussurea costus* roots have been found to exhibit anti-inflammatory hepatoprotective (Cho *et al.*, 2000 and Yaesh *et al.*, 2010) .

Taxonomic classification according to (Zahara *et al.*, 2014).

Kingdom: Plantae

Subkingdom: Viridiplantae

Infrakingdom: Streptophyta

Division: Tracheophyta

Subdivision: Spermatophytina

Infradivision: Angiospermae

Class: Magnoliopsida

Superorder: *Asteranae*

Order: *Asterales*

Family: *Asteraceae*

Genus: *Saussurea*

Species: *S. costus*

Saussurea costus (Falc.) Lipschitz, syn *Saussurea lappa* C.B. Clarke is belonging to *Asteraceae* family, is a Himalayan species that occurs at elevations from 2,700-4,000 m in Kashmir, Lahul Valley in Himachal Pradesh and Garhwal in Uttranchal (Kaul , 1997).

Saussurea costus lipschitz, syn *Saussurea lappa* C.B. clarke is a well known and important medicinal plant widely used in several indigenous systems of medicine for the treatment of various ailments, viz. asthma, inflammatory diseases, fatigue, dyspnea, and difficulty breathing ulcer and stomach problems sesquiterpene lactones have been reported as the major phytoconstituents of this species (Diao *et al.*,2020).

Lappadilactone, lactone cynaropicrin, dehydrocostus, germacrenes were isolated from fresh roots of plant(Taniguchi , 1995). *Candida* species are yeasts that can dangerously cause many types of fungal infections in human body/organs, whereas “Candidiasis” is the general term to categorize the infectious diseases from *Candida* contagion (Ciurea , 2020).

Different pharmacological experiments in a number of *in vitro* and *in vivo* models have convincingly demonstrated the ability of *Saussurea costus* to exhibit anti-inflammatory, anti-ulcer, anticancer and hepatoprotective activities, chronic candidiasis, vaginitis, and endophthalmitis lending support to the rationale behind several of its traditional uses costunolide, dehydrocostus lactone and cynaropicrin, isolated from this plant, have been identified to have potential to be

developed as bioactive molecules due to the remarkable biological activity of *Saussurea costus* and its constituents. It will be appropriate to develop them as a medicine (Pandey , 2007 and Singh *et al* .,2020)

2.5. *Carthamus tinctorius*

Carthamus tinctorius L., widely accepted as Safflower or false saffron, belongs to the Compositae or Asteraceae family. This thistle-like species typically thrives in an arid climate, namely Southern Asia, China, India, Iran, and Egypt It is found with six species in Iran (Shirwaikar *et al.*, 2010 and Zhao *et al.*, 2009).

The other well-known names for safflower are “Zaffer”, “Fake Saffron”, and Dyer’s Saffron” it has been shown that the scavenging activities of safflower petals can produce a range of colors from orange to white with various intensities (Delshad *et al.*, 2018).

Taxonomic classification according to (Bérvillé *et al.*, 2005).

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

Order: Asterales

Family: Asteraceae (Compositae)

tribe : Cardueae (thistles)

subtribe : Centaureinae

Genus: *Carthamus*

Species: *Carthamus tinctorius*

This plant is used for numerous culinary and textile purposes. With the advent of synthetic aniline dyes, it has been mainly grown as an oilseed and birdseed that has some applications in medicinal fields (Bae *et al.*, 2002). More to the point, its oil has high nutritional value and consists of 70% polyunsaturated fatty acid (i.e., linoleic acid), 10% monounsaturated oleic acid, and mere amounts of stearic acid (Kumar and Kumari, 2011).

Safflower remarkably shows purgative, analgesic and antipyretic characteristics, and is useful in patients with poisoning (Asgarpanah and Kazemivash, 2013).

The flowers of *C. tinctorius* are traditionally applied for cardiovascular, cerebrovascular, and gynecological complications. In this regard, shed light on the phytotherapeutic potency of the water extract of *C. tinctorius* for cardiovascular diseases (Zhou *et al.*, 2009).

As for other biological activities linked to its water extract, it is regarded as an anticoagulant, vasodilating antihypertensive, antioxidative, neuroprotective, immunosuppressive, anticancer agent with inhibitory impacts on the synthesis of melanin. Furthermore, it was indicated that safflower is effective for other ailments involving the neurotropic, cardiotropic, hemopoietic, and diaphoretic systems and used clinically for the prevention and treatment of cardiovascular diseases (Punjanon *et al.*, 2004 and Guo *et al.*, 2019).

Concerning the phytochemistry of this plant, pertained studies have pinpointed a number of active constituents, such as flavonoids,

phenylethanoid glycosides, coumarins, fatty acids, and steroids identified from various parts of the plant (Zhou *et al.*, 2009). Currently, a body of pharmaceutical studies has focused on its ethno-pharmaceutical applications in terms of antioxidation, anti-inflammation, and anti-epilepsy (Kruawan and Kangsadalampai, 2006).

2.6. Secondary Metabolites in Plant:

Secondary metabolites medicinal plant are defined as any plant which has compounds that can be used for the therapeutic purpose or which contain precursors of chemo- pharmaceutical synthesis. Throughout the world, plants are used traditionally to treat many ailments, particularly infectious diseases, such as diarrhea, fever, cold as well as for birth control and dental hygiene (Shapiro *et al.*, 2006).

Plants materials are on increasing interest for their applications in pharmaceutical, nutritional and cosmetic application they represent a source of active ingredients known for long times ago by its traditional used for medical purposes also inducing changes in plant metabolic enzyme, subsequently trigger the production of secondary metabolites (Hectors *et al.*, 2014).

Plants can be considered as an origin of natural ingredients useful in medicine and other purposes. Plants are rich in active compounds or secondary metabolites such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are present in their organs such as leaves, flowers, bark, seeds, fruits, root, etc. Extraction processes of these metabolites are related to the difference in solubility of the compounds present in a mixture of solvent. The beneficial action of those phytoconstituents typically come from the

merging or synergic work of these secondary products (Tonhubthimthong *et al.*, 2001 and Ghasemi *et al.*, 2019).

2.6.1 Alkaloids

Alkaloids are generally present in higher plants, particularly in dicots, whereas only a few have been noted in lower plants. The alkaloids can occur in the whole plant or in the specific plant organ. Alkaloids are derived from amino acid mostly contain one or more carbon rings which usually contain nitrogen. The type of Alkaloids and plant families depend upon the position of nitrogen atom in the carbon ring. Alkaloids play important roles in plants as it checks the feeding of herbivores, protects from pathogenic hit, and inhibitions of competitors (Egamberdieva *et al.*, 2017). Plant alkaloids, one of the largest groups of natural products, represent a highly diverse group of chemical entities. Alkaloids encompass an enormous class of approximately 12000 natural products (Saxena *et al.*, 2013).

Many of these compounds possess potent pharmacological effects for example, the well known plant alkaloids include the narcotic analgesics, morphine and codeine, apomorphine (a derivative of morphine) used in Parkinson's disease, the muscle relaxant papaverine, and the antimicrobial agents sanguinarine and berberine. Also several potent anti-cancer drugs have been developed from plant compounds (Salminen *et al.*, 2011).

2.6.1.1. Distribution and Localization of Alkaloids :

Initially defined as nitrogen-containing, basic substances of natural origin and of limited distribution, alkaloids have a complex structure. Their nitrogen atom is part of a heterocyclic system and they possess a

significant pharmacological activity; according to some authors (Chanda and Ramachandra, 2019).

They are found as salts, and it may be added that they are formed biosynthetically from an amino acid these elements characterize what may be referred to as true alkaloids, many authors distinguish, in addition, proto-alkaloids and pseudo alkaloids only rarely do alkaloid-containing plants contain only one alkaloid: sometimes they do contain virtually only one constituent (hyoscyamine from the leaves of belladonna) but, most often (Bribi, 2018) . They yield a complex mixture, which may be dominated by one major constituent it is not uncommon to find several dozen alkaloids in one drug , as a general rule, all of the alkaloids of a given plant have a common biogenetic origin, even if their structures may at first seem quite different, in a given plant, the concentration of alkaloids can vary widely from part to part, and some parts may contain none (Becker, 1996 and Bribi, 2018) .

2.6.1.2 Pharmacological Activities of Alkaloids :

The activity of alkaloids against herbivores, toxicity in vertebrates, cytotoxicity activity, the molecular targets of alkaloids, mutagenic or carcinogenic activity, antibacterial, antifungal, and antiviral properties, and their possible roles as phytoalexins have been tabulated many alkaloids are sufficiently toxic to animals to cause death if eaten (Bednarz and Niehaus ,2019) .

Several alkaloids such as nicotine and anabasine are used as insecticides many alkaloids act on the nervous system, one of two important information systems in animals (Singh and Pandey, 2021).

Plants that contain protoberberine alkaloids are reported to be used as analgesics, antiseptics, sedatives, and stomatics in Chinese folk

medicine, in Indian and Islamic folk medicine such plants are used for bleeding disorders and eye diseases, and antiseptics, sedatives, stomatics, and uterine muscle depressants both quaternary alkaloids and their tetrahydro derivatives possess many substantiated biological and therapeutic effects, e.g. palmatine, jatrorrhizine, and tetrahydropalmatine have been reported to show in vitro antimalarial activity in china, tetrahydropalmatine is used as an analgesic, and has been reported to exhibit bradycardial, hypotensive, and sedative activities (Bribi, 2018).

2.6.2 Flavonoids

Flavonoids form one of the major groups of specialized metabolites, and include over 9,000 compounds (Andersen and Markham, 2005). According to Nomenclature of flavonoids (IUPAC Recommendations, 2017).

The term flavonoid is applied to (1) compounds structurally based on derivatives of a phenyl-substituted propylbenzene having a C₁₅ skeleton, (2) compounds with a C₁₆ skeleton that are phenyl-substituted propylbenzene derivatives (rotenoids), (3) flavonolignans based on derivatives of phenyl-substituted propylbenzene condensed with C₆-C₃ lignan precursors (Rauter *et al.*, 2018)

2.6.2.1. Distribution of Flavonoids:

Flavonoids are estimated that there are over 9,000 flavonoids in the plant kingdom (Andersen and Markham, 2005). Research on flavonoids has shown that they are distributed across the plant kingdom, including angiosperms, gymnosperms, and pteridophytes (Tohge *et al.*, 2013).

The abundance of information about flavonoids in different species allows us to identify which flavonoid subclasses (e.g., chalcones, flavones, flavonols, anthocyanins, and proanthocyanidins) are found in each subgroup of plants flavone and flavanone are found in all plant groups except for hornworts (Agati *et al.*, 2020).

2.6.2.2. Pharmacological Activities of Flavonoids:

One of the very visible functions of flavonoids is in flower pigmentation, and, associated with that, in the attraction of pollinators and UV protection; this topic is exemplified by Dudek and colleagues, who explored the spatial occurrence of flavonoids in Papaver flowers, which present a beautiful model due to their wide-ranging variation in flower colour (Dudek *et al.*, 2016).

The insightful review of the flavone by Jiang and colleagues paints a colourful picture of their biosynthesis and roles in plants, microbes, animals, and their interactions (Jiang *et al.*, 2016). Not only have flavones taken on very specific functions in controlling plant development through their action in cell wall synthesis, but flavones also have activity in allelopathic action against other plants, in interactions with symbiotic mycorrhizal fungi and rhizobia, and in defence against fungal pathogens (Hano *et al.* ,2018).

An important aspect of flavone bioactivity is their structural diversity, which has allowed flavones to interact with a large variety of other molecules, determining their functions in lipid oxidation, DNA, and protein binding. These interactions underlie their multiple effects on human health, where their functions in the control of redox status, enzyme function, lipid peroxidation, inflammation, cell cycle, and cancer

are well established this is further explored in the articles by Navarro and colleagues (Navarro *et al.*, 2017)

2.6.3. Terpenoids:

Terpenoids constitute one of the largest and structurally diverse groups of naturally occurring compounds, terpenoids are a class of natural products derived from mevalonic acid (MVA) which are composed of a plurality of isoprene (C₅) structural units, terpenoids are widely found in nature, with various structures and a wide variety, to date, more than 50 000 terpenoids have been found in nature (SUN *et al.*, 2017).

Terpenoids isolated from plants some terpenoids play an important role in plant growth and development, such as gibberellin, as plant hormones regulates plant development and carotenoids participates in photosynthesis; some terpenoids play a role in the interaction between plants and the environment, such as participating in plant defense systems in the form of phytoalexins and interspecies competition as interspecific sensing compounds (Arimura *et al.*, 2000 and Perveen and Al-Taweel, 2018).

Many volatile terpenoids such as menthol and perillyl alcohol are used as raw materials for spices, flavorings, and cosmetics (Martin, 2003). There are also some terpenoids with important economic value. They are used as pesticides, industrial raw materials, etc., such as pyrethrin and limonoids, which are often used as insecticides. Sesquiterpenes, farnesene and bisabolene, and monoterpenes, pinene and limonene, are recognized as precursor compounds for fuels (George *et al.*, 2015).

2.6.3.1. Distribution of Terpenoid:

Terpenoid biosynthesis usually highlight the large number of terpenoid compounds found in nature, indeed, the structural diversity associated with at least 40,000 compounds makes the class of terpenoids one of the most impressive examples in the divergent evolution of plant chemicals, the evolutionary success of this compound class is in part based on the simplicity of constructing different size molecules according to the isoprene rule recognized by Wallach and Rutzicka in the late nineteenth and mid-twentieth centuries (Kubeczka, 2020).

All terpenoids are derived from the universal five-carbon building blocks, isopentenyl diphosphate (IPP) and its allylic isomer dimethylallyl diphosphate (DMAPP), the prenyl diphosphate intermediates built by condensation of these five-carbon units are used as precursors for the biosynthesis of terpenoids with fundamental functions in growth and development and for the formation of a large number of terpenoid compounds with more specialized roles in the interaction of plants with their environment (Swor *et al* .,2022).

The latter group of terpenoids that is characterized by its tremendous structural diversity as a consequence of divergent biosynthetic gene evolution specialized terpenoids have a long history of being used as flavors, fragrances, pharmaceuticals, insecticides, and industrial compounds, several of which are addressed in this book with the growing need for sustainable production platforms of plant-based drugs and the emerging use of terpenoids in the production of alternative fuels, substantial progress has been made in the engineering of terpenoid biosynthetic pathways in microbes and plants (Lange and Ahkami, 2013 and Kari *et al* .,2018).

2.6.3.2 Pharmacological Activities of Terpenoids:

Terpenoids (or isoprenoids) are the largest group of natural products with extreme diversity. More than 55,000 compounds have been identified (Breitmaier 2006). Some terpenoids are essential in primary metabolism, including photosynthesis (chlorophyll, carotenoids, and plastoquinone), respiration (ubiquinone), developmental regulation in plants (gibberellins and abscisic acid) (Ludwiczuk *et al.*, 2017).

Most terpenoids are produced by plants as secondary metabolites. Isoprene is a volatile molecule emitted from numerous plant species to protect against heat stress. Isoprene enhances membrane integrity for the purpose of heat tolerance (Siwko *et al.* 2007). Because heat can cause leakage of thylakoid membrane and affect the efficiency of photosynthesis (Sharkey *et al.* 2008). Terpenoids have a variety of commercial applications, including pharmaceuticals, nutraceuticals, flavors/fragrances, and industrial chemicals (Ajikumar *et al.* 2008).

Artemisinin is a sesquiterpene produced by the plant *Artemisia annua*. Artemisinin derivatives are the first-line anti-malarial drugs recommended by World Health Organization, the price and availability of artemisinin fluctuate widely due to unstable supply of agricultural materials with recent improvement in synthetic biology and metabolic engineering, the yeast *Saccharomyces cerevisiae* was engineered for commercial production of artemisinic acid, the precursor of artemisinin (Paddon and Keasling 2014).

2.7 Candida

Candida is a fungus Like other fungi, they are non-photosynthetic, eukaryotic organisms with a cell wall that lies external to the plasma membrane there is a nuclear pore complex within the nuclear membrane. The plasma membrane contains large quantities of sterols, usually ergosterol apart from a few exceptions, the macroscopic and microscopic cultural characteristics of the different *Candida* species are similar they can metabolize glucose under both aerobic and anaerobic conditions temperature influences their growth with higher temperatures such as 37°C that are present in their potential host, promoting the growth of pseudohyphae (Pristov and Ghannoum , 2019).

They have been isolated from animals and environmental sources they require environmental sources of fixed carbon for their growth filamentous growth and apical extension of the filament and formation of lateral branches are seen with hyphae and mycelium and single cell division is associated with yeasts (Lehmann, 2010 and Marak and Dhanashree, 2018).

Candida is part of our normal microbial flora on mucosal surfaces, from where it may cause local infections like thrush in the oral cavity and *Candida* vaginitis (Smith et al., 2007). However, in patients with various underlying diseases or host factors *Candida* may cause invasive disease (invasive candidiasis or candidosis), most often as bloodstream infection (*Candida*emia) with or without secondary dissemination to the eyes, liver, spleen, bones, heart valves, central nervous system and so on or as deep-seated candidiasis, such as peritonitis after gastrointestinal surgery the overall mortality associated with *Candida*emia is around 30–40% and depends on the severity of underlying

disease, the *Candida* species involved, and timing and choice of antifungal treatment. The mean additional costs are significant and estimates range from 8000 s in ICU patients colonised (Staniszewska, 2020) .

2.9. Candidiasis

Candidiasis is an opportunistic infection caused by *Candida*, a type of fungi fungi are eukaryotic organisms found in the form of yeasts, molds, or dimorphic fungi *Candida* is a form of yeast candidiasis occurs most commonly as a secondary infection in immunocompromised individuals synonyms of candidiasis include candidosis, moniliasis, and thrush these are common inhabitants in the oral cavity, gastrointestinal tract, vagina penis, or other parts they become pathogenic only when favorable conditions arise (Pramanick *et al.*,2019).

Candida species are responsible for the majority of human infections caused by fungal pathogens members of these species include the most frequent cause of opportunistic infections, *Candida albicans*, the drug-resistant *Candida glabrata*, the new global public health threat and other emerging species such as *Candida tropicalis*, *Candida* *Candida dubliniensis* and *Candida krusei* (McCarty *et al.*, 2021). Existing as a commensal in a large proportion of the human population, *Candida albicans* colonizes the oral, gas- trointestinal, and genital tracts asymptotically (Lionakis and Hohl, 2020).

2.8. *Candida* Species

Candida species are the fourth leading cause of nosocomial bloodstream infections in the United States (Weinstein *et al.*, 2005). *Candida albicans*, *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei*, and *Candida guilliermondii* comprise the

main pathogens responsible for invasive candidiasis (Horn *et al.*, 2009 and Pfaller *et al.*, 2010).

Candida species represent ubiquitous commensal yeasts that constitute part of the normal human skin and gut microbiota *Candida* species can be detected on the mucosal surfaces of approximately 50–70% of healthy humans (Pappas and Lionakis, 2018).

Candida species are common causative agents of superficial and invasive fungal infections worldwide (Antinori *et al.*, 2016). Among NACs, *C. tropicalis* is the first or second most commonly isolated species from patients with candidiasis, especially in tropical regions such as Brazil and Asia (Rodrigues *et al.*, 2017).

2.8.1. *Candida albicans*

Appears in several morphological forms (blastospores, pseudohyphae, and hyphae) blastospores divide asexually by budding During that process, new cell material is formed on the surface of the blastospore (Walker and White, 2017).

The new bud grows from a small selected blastospore, and it is most often located distally from the site of a scar caused by birth, after which the phase of growth begins, after the growth phase ends, the cells divide, whereby the daughter separates from the parent cell by creating a partition (Wilson, 2019).

Candida albicans Chains of elongated yeast cells characterize pseudohyphae, and the shape of hyphae is characterized by branched chains of tubular cells, with no narrowing at the sites of septation (Kornitzer, 2019). Filamentation is enhanced by a temperature higher

than 37 °C, an alkali pH, serum, and high concentrations of CO₂ (Basso *et al.*, 2018).

This transition from a blastospore to a hypha is characterized by the activation of a complex regulatory network of signal paths, which include many transcription factors (Basso *et al.*, 2018). The main difference between yeast and hyphae composition is that the hypha wall has slightly more chitin content than yeast (Garcia-Rubio *et al.*, 2020). The cell wall is made of glucan, chitin, and protein. Its role is to protect the cell from stressful conditions in the environment, such as osmotic changes, dehydration, and temperature changes, and protect the cells from the host's immune defense (Reyna-Beltrán *et al.*, 2019).

It is also responsible for adhesion to the host cell, with adhesion proteins such as Als1-7, Als9, and Hwp1 (Ciurea *et al.*, 2020). Communication of the cell with the outside environment takes place through the cell membrane (Hall, 2015). Sterols in the cell membrane are extremely important, giving the cell stability, rigidity, and resistance to physical stressors (Garcia-Rubio *et al.*, 2020). Ergosterol is the most represented sterol and is characteristic for the cell membrane of fungi, it is synthesized on the endoplasmic reticulum and lipid bodies (Jordá and Puig, 2020). In the cell membrane, there is a phospholipid bilayer containing proteins with the role of receptors, but also some whose role is transport and also signal transduction (Cho *et al.*, 2016).

2.8.2. *Candida tropicalis*

Candida tropicalis is closely related to *C. albicans* based on genomic comparisons, but the natural habitats of these pathogens differ: *C. albicans* is a human commensal organism and is rarely found in the environment, whereas *C. tropicalis* is not only a commensal of the human

oral cavity, but is also present in soil, compost, plants, beaches, and seawater in tropical or subtropical areas (de Barros *et al.*, 2018).

Candida tropicalis is common opportunistic yeast pathogen, capable of causing both superficial and systemic infections in humans. *Candida tropicalis* was first isolated from a patient with fungal bronchitis in 1910 and was initially named *Oidium tropicale* (Zuza-Alves *et al.*, 2017). Since then, this yeast has been found in diverse ecological niches such as in the soil, aquatic environments (both fresh water and marine ecosystems), a diversity of trees and plant debris, as well as from humans and other animals (Wu *et al.*, 2019).

Similar to its broad ecological distribution, *C. tropicalis* is also geographically widely distributed, particularly prevalent in tropical and subtropical regions such as Southeast Asia, Africa, and Central and South America (Xu, 2021). In humans, *C. tropicalis* has been isolated from diverse body sites, from our skin to the oral cavity, the gastrointestinal tract, and the urogenital systems in many healthy hosts, and in patients with systemic infections including the circulatory system and other typically sterile body sites (Pinto *et al.*, 2019).

Among patients with systemic candidiasis, *C. tropicalis* is commonly reported as the second- or third-most common causative agent, especially in those with hematological malignancies (Zuza-Alves *et al.*, 2017).

2.8.3. *Candida dubliniensis*

Candida dubliniensis is a dimorphic yeast that was first described in 1995 in the oral cavity of HIV-infected individuals (Sullivan *et al.*, 1995). Though phenotypically similar to *C. albicans*, *C. dubliniensis* is

thought to be less virulent due to decreased expression of phospholipases, aspartyl proteinases and virulence genes (Moran *et al.*, 2012).

Candida dubliniensis colonizes the oropharynx and respiratory tract of immunocompromised hosts and is increasingly being recognized as an opportunistic pathogen in blood, soft tissue, urogenital, gastrointestinal and most recently, corneal compartments (Khan *et al.*, 2012). However, risk factors for *C. dubliniensis* colonization are poorly studied. *C. dubliniensis* meningitis is exceedingly rare, especially in immunocompetent individuals (Yamahiro *et al.*, 2016).

2.9.1. Vaginal Vulvovaginal Candidiasis:

Vulvovaginal candidiasis (VVC) is a common infectious disease affecting the female genital tract and is caused by *Candida* spp of all the VCC cases, 80–90% are caused by *C. albicans*, and a minority are caused by *C. glabrata*, *C. parapsilosis*, and *C. tropicalis* (Denning *et al.*, 2018).

Vulvovaginal candidiasis is characterized by acute inflammation of the vulva and vaginal mucosa caused by an over-growth of *Candida* spp. that normally colonize the vaginal mucosa, increased adhesion to host surfaces, and host genetic predisposition (Rodríguez *et al.*, 2019). *Candida* vaginitis is associated with a normal vaginal pH (<4.5) signs and symptoms of VVC include white clumpy discharge, burning, redness, swelling, and itching in the vulva and vagina, and dyspareunia (Denning *et al.*, 2018).

Most experts believe that 90% of vaginitis cases are subsequently induced by bacterial vaginosis (BV), *Candida* vaginitis (VVC), and *Trichomonas vaginalis* (TV) (Ilkit and Guzel, 2011). They affect millions of women every year and are closely correlated with several adverse effects, containing preterm labor and delivery pelvic

inflammatory disease (Robinson *et al.*, 2016). Postabortal endometritis etc, such as *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, HPV, HSV-2 and HIV-1 (Brotman *et al.*,2010).However, upon perturbation of barrier integrity and/or host immune responses, the fungus can migrate through the epithelium and access deep-seated anatomical niches to cause infection (Azie *et al.*,2020) .

Medically important infections caused by *C. albicans* can broadly be classified into two subtypes: mucosal and systemic Mucocutaneous surfaces primarily affected by *C. albicans* are the vaginal vulvovaginal candidiasis (VVC) (Felix *et al.*, 2019).

2.9.2. Orthodontic Candidiasis:

Removable orthodontic appliances are a popular tool to move, or retain, teeth during, or after, orthodontic treatment nocturnal wearing of the acrylic removable orthodontic appliances may play a role in the development of oral *C. albicans* infections (Ghazal *et al.*,2019). This could be due to low salivary flow and consequently low pH levels as well as impaired oral hygiene wearing acrylic orthodontic appliances has been reported to be associated with increased proliferation of *Candida* regardless of the host immune system status (Rodríguez *et al.*,2019).

These appliances provide an enhanced environment for *C. albicans* growth as they cover a large area of mucosal tissues for a considerable amount of time every day for a relatively long period the removable appliances protect the yeast from the natural flow of saliva and mechanical removal effects of musculature, the orthodontic acrylic also provides a hydrophobic surface to which *C. albicans* can bind through the hydrophobic effect and van der Waals forces (Grzegocka *et al.*,2020).

The combination of these factors may tip the balance in the wearers of orthodontic appliances to support *Candida* colonization and proliferation and also increase dentine demineralization by enhancing the cariogenic potential of *Streptococcus mutans* containing biofilms (Ghazal *et al.*,2019).

Although the increased colonization of individuals wearing removable dentures and removable orthodontic appliances by *C. albicans* might be expected to be similar the two types of acrylic resin have different physical properties, and different fabrication and polymerization methods that affect the amount of residual monomer, which might affect the surface morphology and *C. albicans* adhesion (Lionakis and Hohl, 2020).

2.10. Varulance Facter and Resistance:

There is substantial geographical, centre-to-centre and unit-to-unit variability in the prevalence of *Candida* spp *C. albicans* continues to be the most prevalent *Candida* sp. causing disease in both adult and paediatric populations, but the past decades have witnessed an increase in diagnoses of non-*albicans* candidaemia in both groups (Algammal *et al.*, 2020).

The prevalence of non-*albicans Candida* spp. in most regions is usually determined by factors such as background antifungal usage patterns in the region, individual patient risk factors and clonal outbreaks, that is, outbreaks involving a *Candida* spp. molecular strain that is unique in a health-care environment, in the United States and northwestern Europe, the second most common species is generally *C. glabrata* in the non-outbreak setting(Castanheira *et al.*, 2016).

Candida glabrata is also generally more-common among individuals of >60 years of age and among recipients of solid organ transplant (McCarty and Pappas, 2016). In Latin America, Southern Europe, India and Pakistan, *C. parapsilosis* and/or *C. tropicalis* are much more frequently encountered than *C. glabrata*. *C. krusei* is the least common of the five major *Candida* spp., and it is most often found among patients with underlying haematological malignancies who have received antifungal prophylaxis with fluconazole (Castanheira *et al.*, 2013). A very worrisome global trend is the emergence of *C. auris* (Chowdhary *et al.*, 2017).

2.10.1. Resistance of *Candida*

in *Candida* spp. is either intrinsic (that is, found in all isolates within a species, such as fluconazole resistance in *C. krusei*) or acquired (that is, found in an isolate from a species that is normally susceptible, such as echinocandin resistance in *C. glabrata*) (Rocha *et al.*, 2019). Echinocandin resistance in *Candida* spp. is emerging, particularly in *C. glabrata*. The target for the echinocandins is the β -D-glucan synthase enzyme, which is important for the cell wall synthesis. This enzyme is encoded by *FKS1* in all *Candida* spp. and is also encoded by *FKS2* in *C. glabrata* (Wu *et al.*, 2019).

Mutations in two hotspot regions (HS1 and HS2) of these genes have been identified as the underlying mechanism for echinocandin resistance, where the level of resistance is dependent on the position of the mutated codon (and the corresponding amino acid), the specific amino acid alteration (which amino acid replaces the original one) and in which species the mutation occurs (Jensen *et al.*, 2014 and Haque *et al.*, 2018).

Acquired azole resistance is less common, and from a mechanistic point of view, it is often more complex, as several mechanisms including *ERG11* mutations, upregulation of the target protein production and efflux pumps often play in concert (Morio *et al.*, 2017).

Although multidrug resistance is a rare phenomenon (Jensen *et al.*, 2015). Mutations in *MSH2*, which encodes a DNA mismatch repair protein, in *C. glabrata* have been found in clinical isolates and have been demonstrated to facilitate selection of resistance to azoles, echinocandins and polyenes *in vitro* (Healey *et al.*, 2016). The mutation of resistance mechanism may explain in part why echinocandin resistance in *C. glabrata* has been significantly linked to prior echinocandin and also fluconazole exposure or resistance, despite the fact that azoles and echinocandins have completely different targets in the cell (Farmakiotis *et al.*, 2014 and Pristov and Ghannoum, 2019).

2.10.2. Fungal Biofilms:

Fungal biofilms are the complex association of hyphal cells which in turn are associated with both abiotic and animal tissues they are important virulence factors and correlated with invasive fungal infection (Borghi *et al.*, 2015). They are the sessile microorganisms that, when attached to the abiotic or biotic surfaces, lead to new phenotypic characteristic features (Lohse *et al.*, 2018). Implantable medical devices are the favorable sites where *C. albicans* form a complex association forming the biofilms, thus becoming responsible for a proportion of clinical candidiasis (Douglas, 2002 and Lagree *et al.*, 2018).

Furthermore, adherence of the fungal cell to the available biomaterial and its relatedness to bloodstream infections might be due to hematogenous spread medical devices provide a perfect niche to yeast

Chapter Two Literature Review

cells because of their structure to chemical properties ranging from hydrophobicity to surface roughness these devices are surrounded by body fluids like urine, blood, saliva, and synovial fluid, which condition them with glycoproteinaceous film (Subbiahdoss *et al.*, 2010).

This acclimatizing film can accord chemical properties entirely different from its origin, the mature biphasic structure of *C. albicans* is promoted by non-specific factors (cell surface hydrophobicity and electrostatic forces) and specific adhesins on the fungal surface recognizing ligands in the conditioning films, such as serum proteins (fibrinogen and fibronectin) and salivary factors (Demuyser *et al.*, 2014).

The extracellular matrix is a critically important feature of biofilms that guards the adherent cells against the host immune system and antifungal agents by forming an extensive structure of the matrix (Borghi *et al.*, 2016). In some of the pioneer works, it was shown that *Candida* species biofilm's matrices increase when highly dynamic flow environments influence biofilm, and its quantity extensively depends upon strain and the species. Moreover, the chemical composition of *C. albicans* ECM suggests that the extracellular matrix is composed of approximately 55% of a combination of glycoproteins with carbohydrates contributing to only 25% of the total composition (Rocha *et al.*, 2019).

Carbohydrates consist largely of α -mannan and β -1, 6-glucan polysaccharides with β -1, 3-glucans making a very little contribution. ECM also consists of 15% of lipids and only 5% of nucleic acids, β -1, 3-glucan plays a major role in azole resilience by specific binding. Additionally, the biofilm is two times thicker than planktonic cells, upon comparison of the chemical composition of planktonic cells with biofilm

cells, it was observed that there is a difference in carbohydrate and β 1, 3 glucan composition (Lohse *et al.*, 2018).

2.10.3. Hemolytic:

Candida species have been recognized as normal flora of human skin, although *Candida albicans* is the most frequently isolated fungal species from human infections, *Candida parapsilosis* has become the second or third most common cause of fungal infections in human (Tavanti *et al.*, 2005 and Furlaneto *et al.*,2018) .

Nowadays, these species have been reclassified as a *C. parapsilosis* species complex comprising three distinct species: *C. parapsilosis*, *Candida orthopsilosis*, and *Candida metapsilosis*, using different molecular methods (Paula *et al.*, 2019). Several factors, such as adherence, persistence, germ tube formation, phenotypic switching, interference with host defense systems, synergism with bacteria, and the production of hydrolases, have been proposed to be *Candida* spp. virulence factors(Yigit *et al.*, 2011 and Pakshir *et al.*, 2018).

The ability of *C. albicans* to form biofilms and adhere to host tissues is important pathogenesis factor. Biofilm formation can act as a reservoir of agents, allow coinfection with other pathogens, promote the persistence of infection, and increase mortality (Mukhia and Urhekar, 2016).The role of exoenzymes as a virulence factor of *C. albicans* has been intensively investigated, but its contribution to the virulence of *C. parapsilosis* species complex remains uncertain (Kocendova *et al.*, 2019). *Candida albicans* is considered to be the most prevalent fungal species present in the human body, where it asymptotically colonizes gastrointestinal and genitourinary tracts and the skin of healthy individuals, it can, however, switch into a virulent form with high

resistance to antimycotics and thereby infect almost every host tissue (Bolognese, 2018).

Immunodeficiency in patients, stress and other external influences can lead to overgrowth of *C. albicans* and its morphological change (as a dimorphic fungus) between yeast-like round cells and hyphae, this morphological switching is known to increase the actual virulence of *Candida* spp. in their hosts (Yang *et al.*, 2018).

The round cells are more susceptible to antimicrobials than are hyphae, and they are less capable of adhering to surfaces others of the fungus's virulence factors include host recognition biomolecules (adhesins), phospholipases, secreted aspartyl proteases and hemolysins that are related to active invasion of host tissue (Bersani *et al.*, 2019). *Candida albicans* thus causes infections ranging from superficial mucosal to hematogenously disseminated systematic candidiasis, e.g. oropharyngeal candidiasis or vulvovaginitis (Uppuluri and Ribot, 2017) .

Most of these infections are strongly related to the great ability of this pathogen to form biofilms, i.e. on medical implants, such as indwelling catheters, artificial joints, pacemakers and central nervous system shunts candidiasis today constitute one of the most frequently occurring nosocomial infections and contribute to almost 40% mortality the formation of *Candida* spp biofilms on these prosthetic devices is one of the major predisposing conditions for the development of systemic candidiasis, antifungal resistance is an intrinsic characteristic of biofilms and its emergence can be detected within minutes to hours of adhesion to a surface (Yang *et al.* 2018).

Chapter Two Literature Review

Echinocandins and lipid formulations of amphotericin B are possible alternative drugs because fungal biofilms remain susceptible to them even after their formation (Mukherjee and Chandra 2004). However, it should be noted that antifungal azoles are still the drugs of choice for the treatment of *Candida* spp. infections rather than echinocandins, and the phenomenon of biofilm resistance represents a major threat for their prescription (Bersani *et al.*, 2019).

Chapter Three

Materials and Methods

Chapter Three..... Materials and Methods

3. Materials and Methods

3.1. Materials

3.1.1. Laboratory Instruments

Instruments and Equipment :

Table (3-1) Instruments and Equipment used in this Study .

| Seq | Equipment | Manufacturing company |
|------------|-------------------------------|------------------------------|
| 1 | Refrigerator | Concord / Korea |
| 2 | Sensitive Balance | Denver / Swizer Land |
| 3 | Micro Pipettes | Gillson instruments / France |
| 4 | Autoclave | Hariyama / Korea |
| 5 | Compound Microscope | Olympus / Japan |
| 6 | Plan Tube | Afco _Dispo (Jordan) |
| 7 | Petri Dishes | Sterial (Jordan) |
| 8 | Incubator | Memmert / Germany |
| 9 | Flask (250-500) ml | Oxfords |
| 10 | Transport Media Swab | India |
| 11 | Sterile Swab Tube | India |
| 12 | Disposable Gloves | TG Medical/Malaysia |
| 13 | Filter Papers (150)mm Whatman | Zhejiang, China |
| 14 | Parafilm | Zhejiang, China |
| 15 | Cloth | Iraq |

Chapter Three..... Materials and Methods

| | | |
|----|-------|------|
| 16 | Plate | Iraq |
|----|-------|------|

3.1.2 Chemical and biological Materials

Table (3-2) Chemical and Biological Materials used in this Study

| Seq | Materials | Industrialization |
|-----|---|-------------------------|
| 1 | Ethano | BDH/England |
| 2 | Chloroform | BDH/England |
| 3 | Acetic acid | BDH/England |
| 4 | Dimso | BDH/England |
| 5 | Clyserol | BDH/England |
| 6 | Gram Stain Kit (4*250)ml R1-Crystal violet Reagent R2-Iodine Reagent (stabilized) R3-Decolorizer Reagent R4-Safranin Reagent | Spectrum/Egypt |
| 7 | Antibiotic (Fluconazole) (Ketoconazole) (Caspofungin) | Sudi corp /Saudi Arabia |

3.1.3 Cuiture Media

Table (3-3) Culiture media used in the Laboratory .

| Saq | Media | Manufacture |
|-----|-----------------|----------------|
| 1 | Mannitol Agar | Hi media-India |
| 2 | Blood Agar base | Hi media-India |

Chapter Three..... Materials and Methods

| | | |
|---|----------------------------|------------------|
| 3 | Mcconky Agar | Hi media-India |
| 4 | Sabouraud Dextrose Agar | Hi media-India |
| 5 | Potato Dextrose Agar | Hi media-India |
| 6 | Chrom Agar Candida | Liofilchem-Italy |
| 7 | Brain Heart Infusion Broth | Hi media-India |
| 8 | Mueller Hinton Agar | Hi media-India |

3.2 Preparation media

The media were shown in Table (3-3) for isolating and diagnosing fungi and yeasts according to the instructions of the manufacturer installed on the packages or according to the references of the scientific references and pH modification where necessary using the KOH and HCl. All the media were sterilized in a temperature of (121 C°) , under pressure of 15 PSI for 15 minutes and the most important of these media are :

3.2.1 Manitol Agar

Dissolve 111gms in 1000ml of distilled water. Gently heat to boiling with gentle swirling and dissolve the medium completely. Sterilize by autoclaving at 15 psi (121°C) for 15 minutes. Cool to 45-50°C. If required, aseptically add 5% v/v Egg yolk emulsion (TS 002) after sterilization. Mix well and pour into sterile Petri plates.

3.2.2 Blood Agar

This medium is prepared by sterilized SDA medium by autoclave. After cooled to 50°C and add 5% v/v sterile human blood, is mixed well and poured into sterile petri plate. This medium is used to determine the viability of yeasts to hemolysis.

3.2.3 Mcconky Agar

Suspend 51.5 gram in 1 litter of distilled water, Heat until completely autoclave at (121°C) for 15 minutes .

3.2.4 Sabouraud Dextrose Agar

These prepared by suspending 65 g of SDA medium in 1000 ml of D.W and regulating the pH to 6.5 according to the instruction of the manufacturers. In an autoclave unit (in the case of yeast cultivation) 0.05 g of chloramphenicol and 0.05 g of streptomycin antibiotics were then added and sterilized, and in the case of fungi cultivation, without streptomycin. Then, this medium was distributed in 9 cm petri dishes.

3.2.5 Potato Dextrose Agar

Suspend 39.0 grams in 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Steriliz autoclaving at 15 Ihs pressure (121°C) for 15 sterile Petri plates or tubes as desired in specific work, when pH 3.5 is required, acidify the medium with sterile 10% HCl acid. The amount of acid required for 100 ml of sterile. cooled medium i approximately 1 ml. Do not beat the medium after addition the acid

3.2.6 Chrom Agar Candida

The chrom agar is prepared by suspending 47.7 g of chrom agar in 1000 ml of D.W. and heated to the point of effervescence (for yeast cultivation), as instructed by the manufacturers. It was then poured into a plastic 9 cm petri dishes. The media is used for researching and diagnosing the Candida spp. Their appearance is based on color the chrom agar is prepared by suspending 47.7 g of chrom agar in 1000 ml of D.W. and heated to the point of effervescence (for yeast cultivation),

as instructed by the manufacturers. It was then poured into a plastic 9 cm petri dishes. The media is used for researching and diagnosing the *Candida* spp. Their appearance is based on color (Nadeem *et al.*, 2010).

3.2.7 Mueller Hinton Agar (MHA):

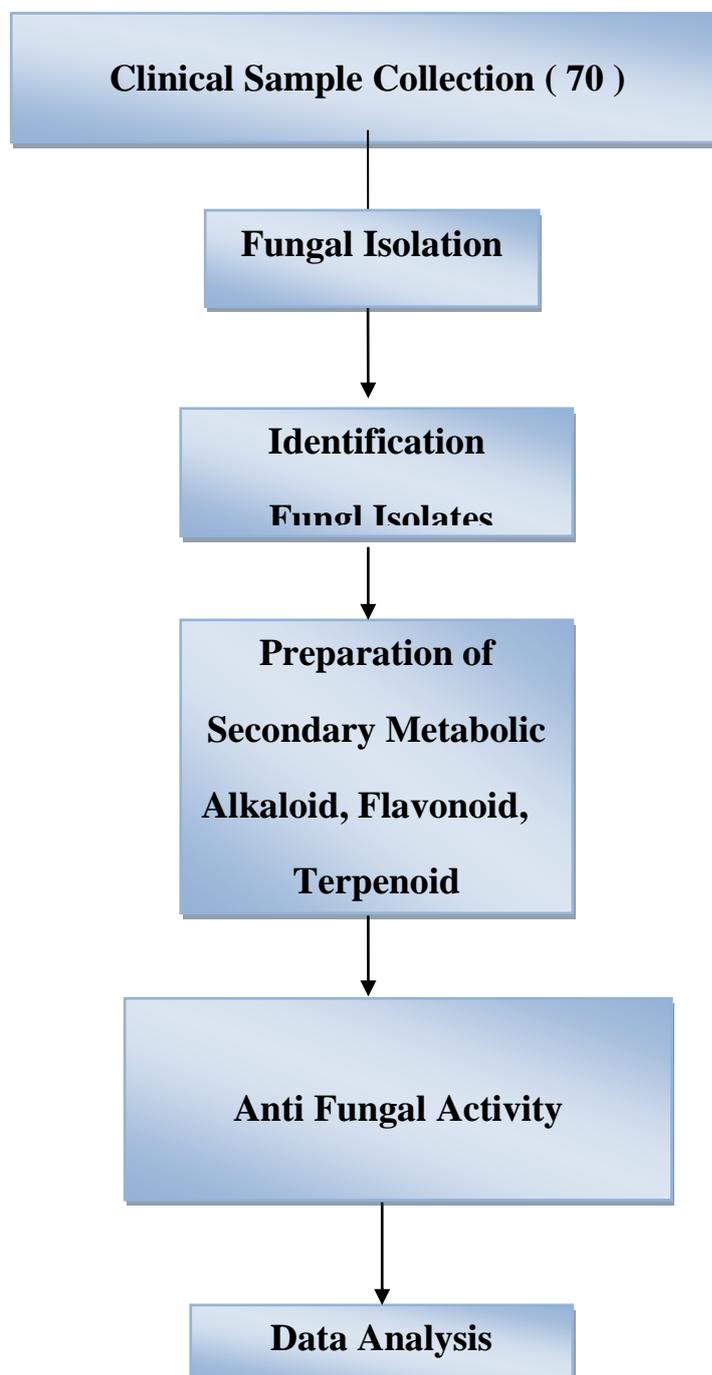
Suspend 38 gm of the medium in one liter of distilled water. Heat with frequent agitation and boil for one minute to completely dissolve the medium. Autoclave at(121°C) for 15 minutes. Cool to room temperature Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth. Allow to cool to room temperature, Check for the final pH 7.3 ± 0.1 at 25°C. Store the plates at 2-8 °C .

3.2.9 Brain Heart Infusion Broth

This medium prepared for the need data by melting 37 g of Distiller water (D.W.) into 1000 ml and heated to boiling to dissolve completely, as well adding chloramphenicol and streptomycin to make the fungal growth medium more competitive and preventing the growth of bacteria, sterilize at (121°C) for 15 minutes (Washington, 2012) BHI broth is a useful resource to encourage extensive types of microorganisms. As it is an extremely nutritious medium. BHI is the anaerobic yeasts, molds and bacteria selection method (Downes and Ito, 2001). This medium is useful for the grow for stored wide range of microorganisms.

3.3 The Methods

3.3.1. Study Design



3.3.2. Specimens Collection

In this study, 70 specimen (212) isolates of vaginal infections were collected from married women of different ages Those who attended the private women's clinic in the Great Prophet complex in Babylon Governorate And 45 specimen (53) isolates from orthodontic people who of different ages the age group .

3.3.3. Identification of Candida

3.3.4. Biofilm formation test

Colonies of yeasts isolations are inoculated in saline and incubated overnight at 37°C. 0.5 ml of saline suspension which is added into screw capped conical polystyrene tubes containing 50 ml of Sabouraud dextrose broth enhanced by glucose (the ending concentration of 8%) and the tubes are incubated in 35°C for 48 h. After incubation the broth from the tubes is aspirated gently using Pasteur pipette the tubes are washed twice with distilled water and stained with 2% safranin the stain is decanted after 10 minutes the tubes are rinsed with distilled water to remove excess stain. Presence of visible adhesive film on the wall and at the bottom of the tube indicated biofilm formation. Ring formation at the liquid interface was not considered as an indication of biofilm production (Deorukhkar and Saini, 2013).

3.3.5. Hemolysis test

Viability of yeasts to hemolysis is determined by inoculated the blood agar medium with yeasts isolates, then incubate in 37°C to 48 h and the positive result is the formation of a clear halo about developing the colony (Sachin *et al.*, 2012).

4.4 Plant Material

4.4.1. Carnation (*D. caryophyllus* L.) flower buds had been purchased from the local markets, identified based on the taxonomic features by a botanist. Flower buds of this plant were cleaned, dried, and kept according to (Harborne *et ai*.,1975).

4.4.2. The roots of (*Saussurea costus*), had been purchased from local markets, identified and classified according to (Table: 1). Roots of this plant was cleaned, dried, and kept according to (Harborne *et ai*.,1975).

4.4.3. Safflower seeds (*Carthamus tinctorius* L), had been purchased from local markets, identified based on the taxonomic features in Iraqi Flora Seeds of these plants were cleaned, dried, and kept according to (Harborne *et ai*.,1975).

4.5 Extraction of secondary metabolites

4.5.1. Alkaloid determination using method According to (Harborne , 1973)

(5 gm) of the sample was weighed into a 250 ml beaker and 200 ml of 10 % acetic acid in ethanol was added and covered and allowed to stand for 4 hrs . This was filtered and the extract was concentrated on a water bath to one - quarter of the original volume . Concentrated ammonium hydroxide (NH_4OH) was added drop wise to the extract until the precipitation was complete . The whole solution was allowed to settle and the precipitated was collected and washed with dilute 1 % ammonium hydroxide and then filtered . The residue is the alkaloid , which was dried and weighed .

4.5.2. Flavonoid Determination by the method according to(Boham and Kocipai Abyazan ,1994) : -

(10 gm) of the plant sample was extracted repeatedly with 100 ml of 80 % aqueous Methanol at room temperature . The whole solution was filtered through whatman filter paper No 42 (125 mm) the filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight .

4.5.3. Extraction of Crude Terpenoid Compounds According to (Harborne , 1984).

(10 gm) of plant powder for each leaves and fruits were extracted by 200 ml of Chloroform in Soxhlet apparatus for 24 hours for extraction the solvent then evaporated in rotary evaporator . The samples were placed in clean dark vials and kept in refrigerator at 4 ° C until use .

4.5.4. Anti-candidiasis Efficacy

The anti-candidiasis activity of the secondary metabolites compounds extracted from the flower buds of (*D. caryophyllus* L.) , root of (*Saussurea costus*) and seeds of (*Carthamus tinctorius* L.) was tested against the isolates of *Candida* species by using agar-well diffusion method wells were made by using cork porer (6mm) in diameter. Dimethyl sulfoxide 10% (DMSO) was used as a negative control and Fluconazole antibiotic as a positive control.

4.5.5. Statistical analysis : All data of treatments were dictated by three replicates. Data were subjected to an analysis of variance by using SPSS 16.0 program, a completely randomized design was used and least significant difference (L.S.D) was performed at $P \leq 0.05$.

Chapter Four

Results and Discussion

4. Results and discussion

4.1. Distribution of age, gender among studied group

In this study, 70 specimen (212) isolates of vaginal infections were collected from married women of different ages who attended the gynecological advisory clinic in the maternity hospital. And 45 specimen (53) isolates from orthodontic people who of different ages the age group (20 – 29) recorded the highest rates 50% of vaginal infection, followed by the age group (30 – 39) which was 31.4% as shown in table (4-1)

The majority of women, according to scientists, have candida in their vaginas without exhibiting any symptoms. If the vaginal environment changes to favour Candida growth, an infection may result hormones, medications, or immune system changes can all cause infection (Sobel,2007). Most females experience vaginitis at least once in their lifetime(Omosa-Manyonyi *et al.*,2022). As one of the most commonly reported vaginal infections, the condition accounts for more than 10% of cases in health care clinics (Loveless *et al.*2018).

Vaginitis generally refers to inflammation of the vaginal wall, with several infectious and noninfectious triggers responsible for this inflammation. Bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonal vaginitis are the most common vaginal infections (Paladine and Desai ,2018). Lactobacillus spp. constitute over 95% of vaginal bacteria and are the predominant microorganisms (Saraf *et al.*,2021).

They are believed to protect the vagina against infection, in part by maintaining an acidic pH and ensuring a hydrogen peroxide environment in the vaginal atmosphere (Onderdonk *et al.*,2016). BV is the most

common cause of abnormal vaginal discharge, is mediated by disturbances in vaginal microbiota and may be temporary or permanent as well as *Candida* species, in particular *Candida albicans*, are part of the normal vaginal microbiota; however, its presence is often asymptomatic thus, a VVC diagnosis requires the comprehensive laboratory investigation of all symptoms (Farhan *et al.*,2019).

Observed that 24% of females (124/516) had mixed infections mixed *Candida* species infections with BV were identified in 108 cases (21%), also, GBS coinfection with *Candida* species was identified in 10 cases 1.93% (Mahakal *et al.*,2013). Found that among 272 females included in their study, the commonest age-group with vaginal discharge was 20–40 years (83%), among whom only 21% of females were pregnant. Similar results were reported in an Indonesian descriptive study (Ocviyanti *et al.*,2010) comprising 492 women, where the average age was 30.9 ± 0.27 years.

Table (4-1) History of Patient with Vaginal Infection

| Characteristics | | No. of patient's specimen (70) | Percentage of patient's specimen % |
|-------------------|--------|--------------------------------|------------------------------------|
| Gender | Male | 0 | 0 |
| | Female | 70 | 100 |
| Age (years) | 20-29 | 35 | 50 |
| | 30-39 | 22 | 31.4 |
| | 40-49 | 11 | 15.7 |
| | 50-59 | 2 | 2.9 |
| Total | | 70 | 100 |
| Chronic disease. | | 0 | 0 |
| Taking antibiotic | | 0 | 0 |

In the case of infections that occur in **orthodontics**, it was noted that the ratio of infection was **48.9%** in males, while in females it was **51.1%** and the rate of infection was 51.1% in the age group (**20-29**) compared to the rest groups as noted in table (4-2)

Treatment with orthodontic appliances promotes *Candida* yeast colonization, which is variable over time in terms of strain and species, with dominance of *C. albicans*, and without increased biofilm-forming activity. Even though *Candida* is a part of the normal oral microbiota, being found in 17–75% human population, it can often be the cause of oral mycoses, especially in immunodeficient patients (van Wyk and Steenkamp, 2011).

The most common etiological factor of oral candidosis is *Candida albicans*. However, *C. tropicalis*, *C. glabrata*, *C. parapsilosis*, *C. krusei* sometimes occur with high prevalence, especially in susceptible patients such as diabetics (Rindum,1994; Hibino *et al.*,2009). All these species have a great ability to form biofilms, especially with oral gram positive bacteria (van Wyk and Steenkamp, 2011).

Table (4-2) History of Patient with Orthodontic Infection

| Characteristics | | No. of patient's specimen (45) | Percentage of patient's specimen % |
|-------------------|--------|--------------------------------|------------------------------------|
| Gender | Male | 22 | 48.9 |
| | Female | 23 | 51.1 |
| Age (years) | 10-19 | 19 | 42.2 |
| | 20-29 | 23 | 51.1 |
| | 30-39 | 3 | 6.7 |
| Total | | 45 | 100 |
| Chronic disease. | | 0 | 0 |
| Taking antibiotic | | 37 | 82.2 |

4.2 Isolation and identification

A total number of bacteria and yeast colonies are 212 isolates from vaginal infections and 53 isolates from Orthodontic infections the number and and yeast species isolated from are summarized in tables (4-3 and 4-4). In the case of vaginal infections, the bacterial infections were 77.8 % (G+ve bacteria were 52.4% and G-ve bacteria 25.4%) were while the yeast infections were 22.2%.

In the orthodontics, the proportion of bacterial infections was 69.8% (G+ve bacteria was 47.2% and G-ve bacteria 22.6%) whereas the incidence of yeasts was 30.2%. The distribution of infectious isolates in vaginal and orthodontics infections were in fig (4-1 and 4-2).

The results of table (4-5) represent the numbers and percentages of *Candida* isolates isolated from vaginal **15.1%** and orthodontic **30.2%** infections which represent the positive for candida infections .

Vaginal complaints are common. Vulvovaginitis, or inflammation of the vulva and vagina, is most commonly secondary to infectious agents in reproductive-aged women. Candidal vulvovaginitis is responsible for about one-third of cases(Buggio *et al.*,2019),(Ahangari *et al.*,2019).

Candidal vulvovaginitis is caused by inflammatory changes in the vaginal and vulvar epithelium secondary to infection with *Candida* species, most commonly *Candida albicans*. *Candida* is part of the normal flora in many women and is often asymptomatic. Therefore, candidal vulvovaginitis requires both the presence of candida in the vagina/vulva as well as the symptoms of irritation, itching, dysuria, or inflammation (Farhan *et al.*,2019).

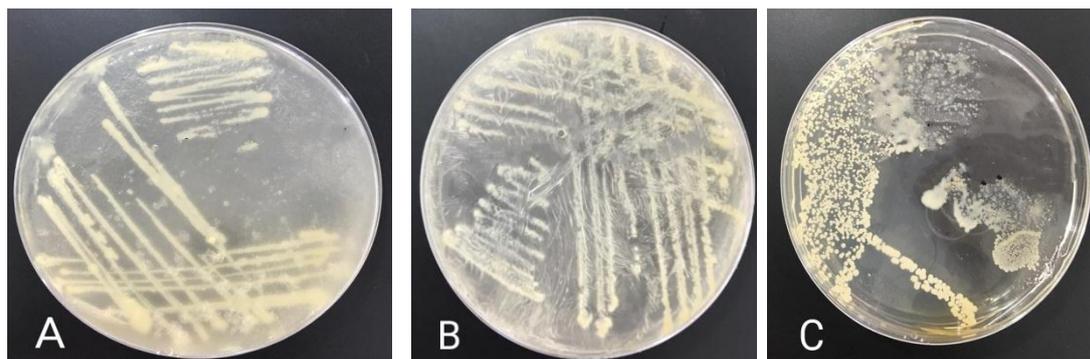


Fig. (4-1): Colonies color of *Candida* species on Dextrose agar medium at 30°C for 24-48h ,A. *Candida albicans* , B. *C. tropicalis*, C. *C. dubliniensis* .

Table (4-3) The frequency of type of infections according to the specimen's type

| isolates | No. among vagina | % of frequency % | No. among Orthodontic | % of frequency % |
|--------------|------------------|------------------|-----------------------|------------------|
| bacteria | 165 | 77.8 | 37 | 69.8 |
| yeast | 47 | 22.2 | 16 | 30.2 |
| Total | 212 | 100 | 53 | 100 |

Table (4-4) The frequency of type bacteria and yeast according to the specimen's type

| isolates | No. vagina specimen | % of frequency % | No. Orthodontic specimen | % of frequency % |
|---------------|---------------------|------------------|--------------------------|------------------|
| G+ve Bacteria | 111 | 52.4 | 25 | 47.2 |
| G-ve Bacteria | 54 | 25.4 | 12 | 22.6 |
| yeast | 47 | 22.2 | 16 | 30.2 |
| Total | 212 | 100 | 53 | 100 |

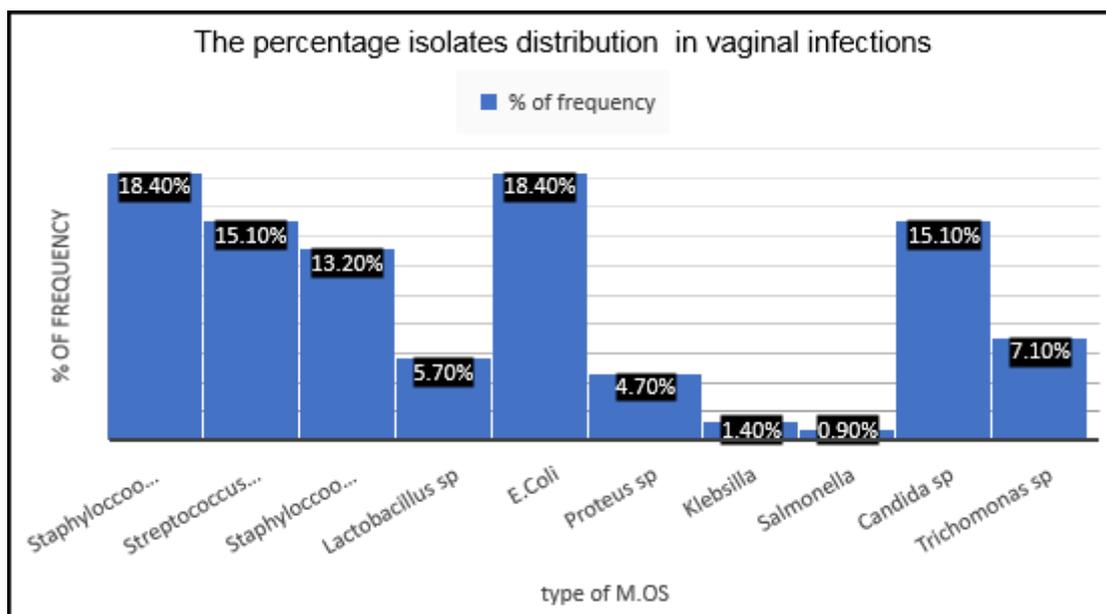


Fig (4-2) The distribution of infectious isolates in vaginal infections

Table (4-5): Distribution Candida spp infections according of specimen's type

| Clinical specimens | No. specimens | No. +ve specimens | % of +ve specimens | No. -ve specimens | % of -ve specimens | Total |
|--------------------|---------------|-------------------|--------------------|-------------------|--------------------|-------------|
| Vagina | 212 | 32 | 15.1% | 180 | 84.9% | 100% |
| Orthodontic | 53 | 16 | 30.2% | 37 | 69.8% | 100% |
| Total | 265 | 48 | 18.1% | 217 | 81.9% | 100% |

4.2. Identification of candida spp

The current study proved the return of 48 isolates to Candida spp by studying some of its characters and biochemical assays are as follows:

4.2.1. Identification by CHROME agar medium

This test is helpful in the laboratory diagnosis of yeast because CHROM agar Candida includes enzymatic chromogenic substrates, which when combined with specific enzymes generated by the varieties

of *Candida* when they grow on this medium, result in varied colours depending on the *Candida* species (Murray *et al.*, 2005). It is a quick test to diagnose the *Candida* species; colonies' development is visible within 24 to 48 hours. Identification of *Candida* species and their proliferation; When compared to conventional techniques, it has the advantages of being technically easy, quick, and affordable. (Vijaya *et al.*, 2011).

The colonies tested in this experiment generate a variety of colours on chrom agar media. On this media, our findings show that *C. tropicalis* colonies create metallic blue hue, whereas *C. albicans* produce pale green. while *C. dubliniensis* colonies produce dark green color on CHROM agar media, fig (4-4). The result of the current study were considered as with (Nadeem *et al.*, 2010; Vignesh Kanna *et al* 2017).

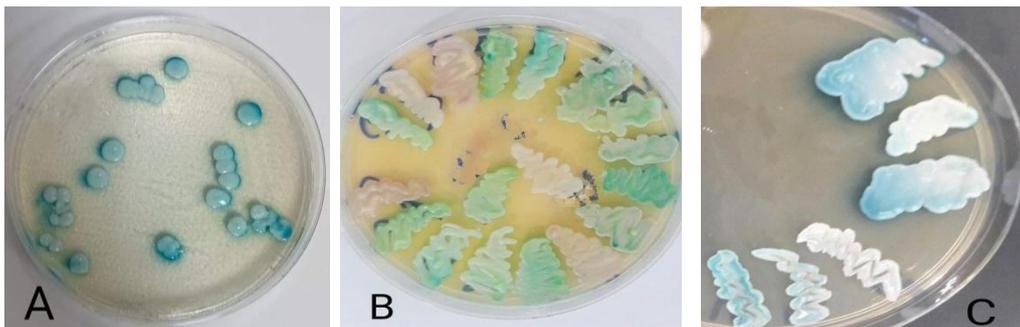


Fig. (4-4): Colonies color of *Candida* species on CHROM agar medium at 30° C for 24-48h . ,A. *C. albicans* ,B. *C. dubliniensis*, c. *C. tropicalis*) .

4.2.2. Identification by Vitik2 System

Vitik-2 system was used in this study in order to diagnose the *Candida* spp . All of the 48 isolates of *Candida* were diagnosed using the Vitik2 System as shown in figures (1,2&3) in Appendix.

Candida vulvovaginitis, or vulvovaginal candidiasis (VVC) It is one of the most common manifestations of *Candida* infection, estimated to affect approximately 75% of women at some point in their lifetime.

Furthermore, over 5% of women suffer at least four episodes of VVC in a year, and this is referred to as recurrent vulvovaginal candidiasis (RVVC) (Brown *et al.*,2012)

Table (4-6): frequency of candida spp in Vaginal and Orthodontic infections

| Candida spp | Vaginal infections | | Orthodontic patients | |
|------------------------|--------------------|----------------|----------------------|----------------|
| | No. of specimen | % of frequency | No. of specimen | % of frequency |
| <i>C. albicans</i> | 17 | 53.1% | 16 | 100% |
| <i>C.tropicalis</i> | 9 | 28.1% | 0 | 0 |
| <i>C.dublinsiensis</i> | 6 | 18.2% | 0 | 0 |
| Total | 32 | 100% | 16 | 100% |

4.1.3. Evaluation of some virulence factors of Candida spp

1. Hemolysis

The result of the study shows the ability of all yeast under study on blood figure (4-5). The *Candida albicans* were given positive result through halo around the developing colonies in the blood agar media.



Fig (4-5) Hemolysin production of candida spp species blood agar medium on 37°c for 48 h, a. Candida albicans ,b. C. tropicalis, c. C. dubliniensis) .

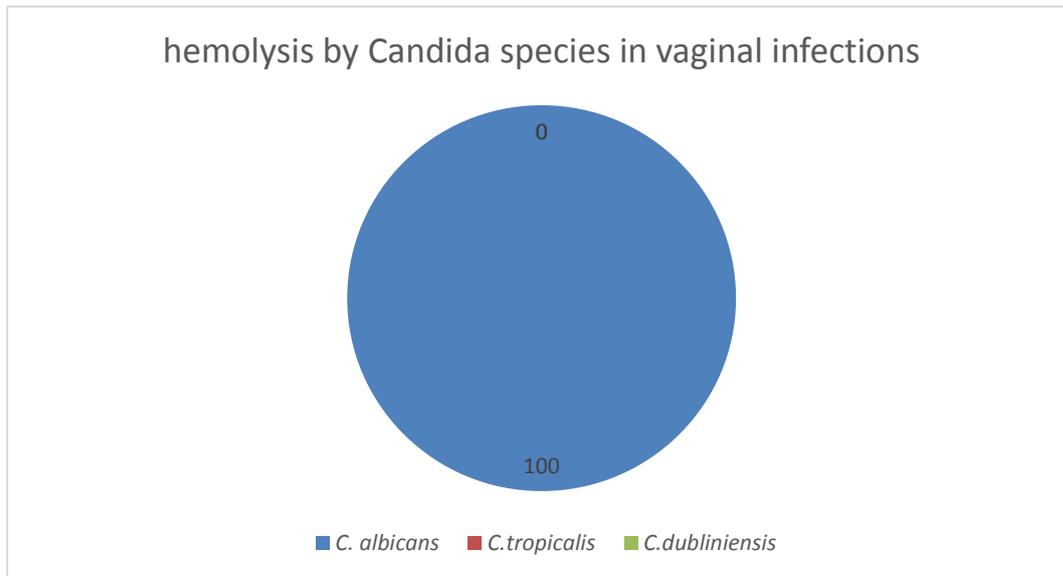


Fig (4-6): hemolysis by Candida species in vaginal infections

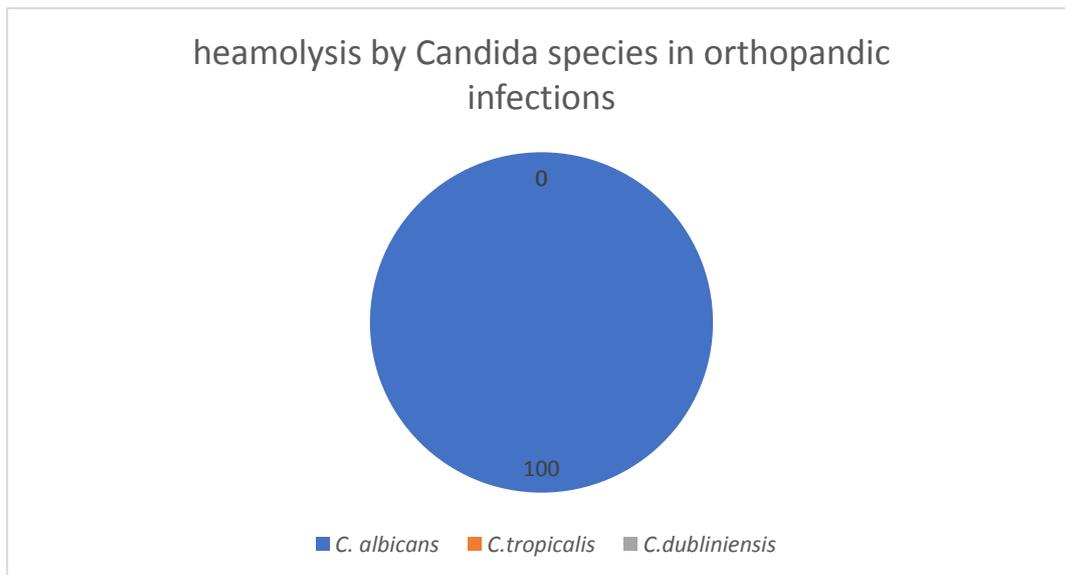


Fig (4-7): hemolysis by Candida species in orthopedic infections

2. Formation of biofilm

Biofilm is essential for the growth of microbes that cause a wide range of clinical infections in humans, as well as the progression of clinical infections. The result of the biofilm test showed that the yeast

isolates have ability to form biofilm and in different proportion as *C. albicans* has ability to the formation of biofilm as well as, *C. dubliniensis* is strong (++) as in figure (4-6). Report that all type of *candida* have the ability to from biofilm and the biofilm is an essential form of microbial growth and is responsible for a wide range of clinical infection of human And some of medically important fungi are form biofilm including candida (Finkel and Mitchell, 2011).



Fig. (4-8): A. Showing some biofilm readings,B. howing the highest biofilm reading and the least reading, C. hows biofilm formation on the walls of the tube.

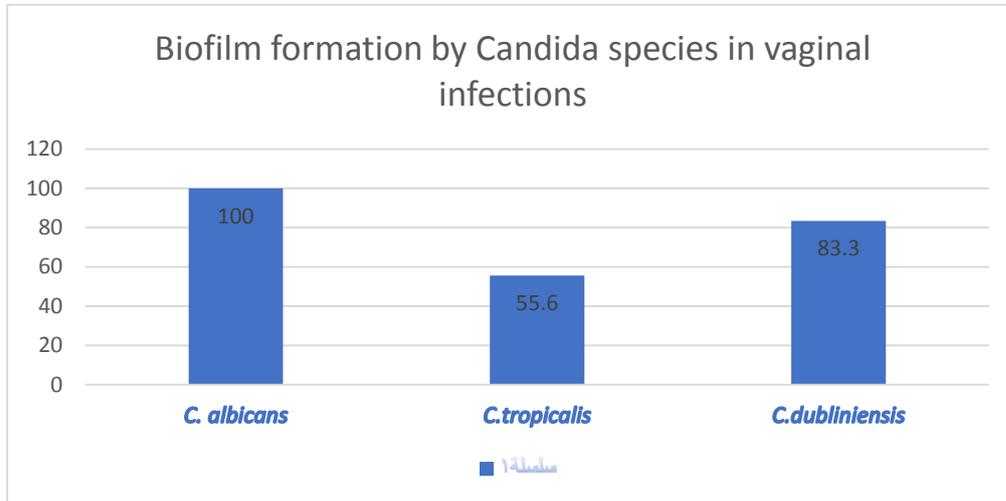


Fig (4-9):Biofilm formation by Candida species in vaginal infections

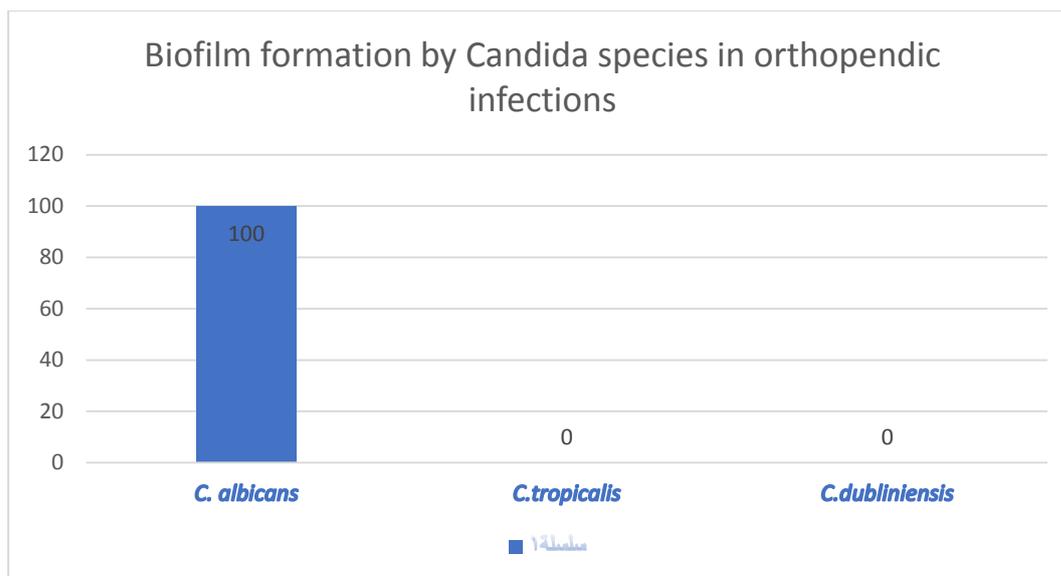


Fig (4-10): Biofilm formation by Candida species in orthopedic infections

3.4. Antifungal activity of three plants (*Dianthus caryophyllus* Saussurea costus and L. ,*Carthamus tinctorius*) against some candida spp.

4.3.1. Antifungal activity of *Dianthus caryophyllus* L. against candida spp

The results of antifungal activity of the crude Terpenoid compounds extracted from the flower buds of (*D. caryophyllus* L.) against *Candida* species isolated from different clinical samples such as mouth and vagina are presented in table (4-7) the antifungal activity of Terpenoid secondary metabolites with three concentrations (25, 50, and 100mg/ml) was screened by agar well diffusion methods. The results revealed that, the crude Terpenoid compounds extracted from the flower buds of (*D. caryophyllus* L.) showed significant reduction at $P \leq 0.05$ in the growth of *Candida* species. Growth inhibition represented by zone of inhibition ranging from (24 ± 1.00 mm in 25 mg/ml, 32 ± 1.00 mm in 50 mg/ ml, and 38 ± 1.00 mm in 100 mg/ml) (Figure: 1 in 4-10), compared with negative control representative by 10% DMSO and positive control representative

by Fluconazole 50mg/ml where inhibition zone was (0.00 mm for negative control and 35± 1.00mm for positive control). On the other hand, the crude Flavonoid compounds showed 19± 1.00mm of zone of inhibition at (25 mg/ml) and 28± 1.00mm at (50 mg/ml), and 37± 1.00mm at (100 mg/ml) concentration (table 4-8), Thus, it differed significantly compared to the negative and positive control treatment (Figure: 2 in fig 4-10).

Table (4-7) Anti-candidiasis activity of the crude Terpenoid compounds extracted from *Dianthus caryophyllus* L. flower buds

| Concentrations (mg/ml) | Terpenoid compounds |
|---------------------------|---------------------|
| | Inhibition zone % |
| Negative Control | 0± 0.00 |
| 25 mg/ml | 24± 1.00 |
| 50 mg/ml | 32± 1.00 |
| 100 mg/ml | 38± 1.00 |
| Positive Control | 35± 1.00 |
| L.S. D | 1.62 |
| *Mean± standard deviation | |

Table (4-8) Anti-candidiasis activity of the crude Flavonoid compounds extracted from *Dianthus caryophyllus* L. flower buds

| Concentrations (mg/ml) | Flavonoid compounds |
|---------------------------|---------------------|
| | Inhibition zone % |
| Negative Control | 0± 0.00 |
| 25 mg/ml | 19± 1.00 |
| 50 mg/ml | 28± 1.00 |
| 100 mg/ml | 37± 1.00 |
| Positive Control | 35± 1.00 |
| L.S. D | 1.60 |
| *Mean± standard deviation | |

In the same context, the crude Alkaloid compounds showed significant activity at three concentrations (25, 50, and 100 mg/ml) compared with negative control against *Candida* species isolated from different clinical samples Table (4-9), The highest zone of inhibition 25 ± 1.00 mm was recorded at 100 mg/ml and 21 ± 1.00 mm was recorded at 50 mg/ml (figure:in fig 4-10 3), While the highest zone of inhibition in the crude Terpenoid compounds was reached up to 38 ± 1.00 mm at 100 mg/ml concentration and the highest zone of inhibition in the crude Flavonoid compounds was reached up to 37 ± 1.00 at 100 mg/ml concentration.

Table (4-9) Anti-candidiasis activity of the crude Alkaloid compounds extracted from *Dianthus caryophyllus* L. flower buds

| Concentrations (mg/ml) | Alkaloids compounds |
|--|---------------------|
| | Inhibition Zone/mm |
| Negative Control | 0 ± 0.00 |
| 25 mg/ml | 14 ± 1.00 |
| 50 mg/ml | 21 ± 1.00 |
| 100 mg/ml | 25 ± 1.00 |
| Positive Control | 35 ± 1.00 |
| L.S. D | 1.62 |
| *Mean \pm standard deviation | |

The Terpenoid and Flavonoid compounds were the most effective compared to the Alkaloid compounds, Figure (4-11), Terpenoids and Flavonoid showed significant superiority at ($P \leq 0.05$) over the Fluconazole antibiotic (35 ± 1.00 mm).



Figure 1: Anti-candidiasis activity of the crude Terpenoid compounds extracted from *D. caryophyllus* L. Flower buds at 50, and 100 mg/ml

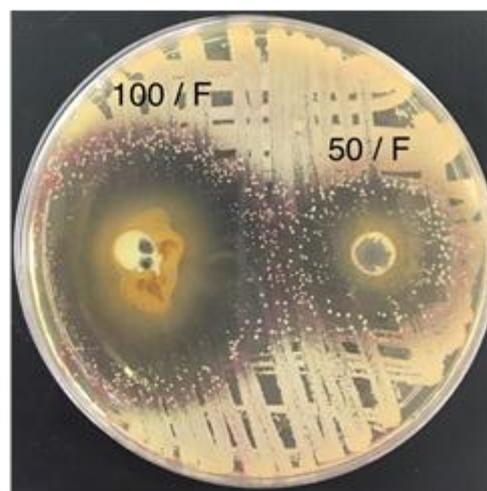


Figure 2: Anti-candidiasis activity of the crude Flavonoid compounds extracted from *D. caryophyllus* L. Flower buds at 50, and 100 mg/ml

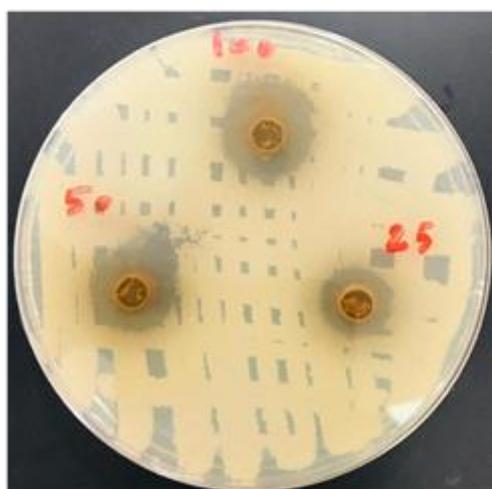


Figure 3: Anti-candidiasis activity of the crude Alkaloid compounds extracted from *D. caryophyllus* L. Flower buds at 25, 50, and 100 mg/ml

Figure (4-11) Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml of *Dianthus caryophyllus* L. flower buds against *Candida* species

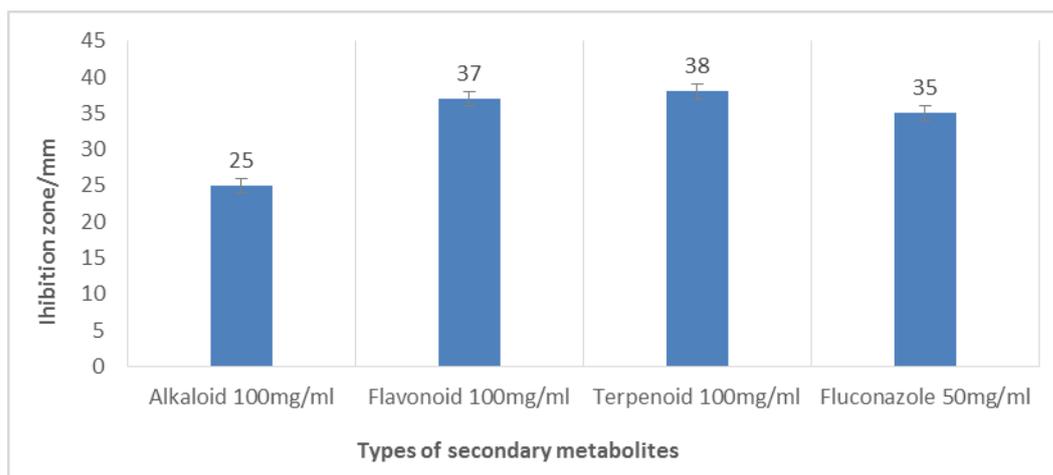


Figure (4-12) Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml against *Candida* species, LSD= 1.82

Emergence of multi-drug resistance in human and animal pathogenic bacteria and fungi like *Candida* species as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. Secondary metabolites extracted from different active parts of numerous medicinal plants such as (*Lactuca serriola* leaves; *Lepidium sativum* leaves; *Myrtus Communis* leaves; *Cassia senna* leaves; *Ricinus communis* leaves; *Cassia didymobotrya* leaves; *Melia azedarach* leaves; *Dianthus caryophyllus* flowers bud; and *Salvia hispanica* seeds), possess ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples (Hussein *et al.*, 2020).

Reported that, phytochemical compounds extracted from the unicellular primitive plant like *Chlorella vulgaris* possess ability of antibacterial counter to pathogenic bacteria. (Kamal, *et al.*, 2019) Used phytochemical compounds extracted from *Hibiscus sabdarifa* for controlling *E. coli* and *Proteus* sp (Kamal, *et al.*, 2020).

Used phytochemical compounds extracted from of *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa*. (AL-Masoodi *et al.*, 2020). Used phytochemical compounds extracted from *Boswellia*

carteri and *Curcuma longa* for controlling *Fusarium* spp. isolated from seeds of maize(Sharara,*et al.*,2020).

Used terpenoids compounds extracted from *Carthamus. tinctorius* seeds and flavonoid compounds extracted from *M. Communis* leaves against *Aspergillus* species isolated from stored medicinal plant seeds. Secondary metabolites represented by Alkaloids and Flavonoids compounds extracted from *M. Communis* leaves respected a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens (Hussain,*et al.*,2021)

Used *Callistemon viminalis* leaves extracts for controlling isolates of Urinary Tract Infections. Phytochemical studies showed that of *D. caryophyllus* contained triterpenes, alkaloids, and coumaruns *D. caryophyllus* grown in Egypt contained four chemical groups: monoterpene hydrocarbons 19.59% (tricyclene 0.17%, α -pinene 2.05%, camphene 0.98%, β -pinene 3.11%, phellandrene 3.52 %, P-cymene 3.32%, limonene 4.91, γ -terpinene 1.53%); oxygenated monoterpene 26.71% (elemol 5.51%, citronellol 1.11%, bornyl acetate 3.12%, eugenol 15.29%, methyl eugenol 1.68%); sesquiterpenes hydrocarbons 12.83% (γ -cadinene 4.12%, calamene 8.71%) and various compounds 20.97% (benzyl benzoate 14.12%, benzyl salicylate 6.85%), Reported that *Dianthus caryophyllus* contained triterpenes, alkaloids, coumaruns, and volatile oil, and the plant possessed anticancer, antiviral, antibacterial, antifungal, insecticidal, repellent, antioxidant, reno-protective, anesthetic and analgesic effects. The flavone datiscetin (3, 5, 7, 2'-tetrahydroxyflavone), presence in *Dianthus caryophyllus* exhibited an appreciable fungitoxic activity towards *Fusarium oxysporum* (Salem,*et al.*,2014).

In contrast, natural bioactive compounds extracted from medicinal plants make their effects by many mechanisms, terpenoids and flavonoids

make their effects by disruption of microbial membranes and Polypeptides embarrassment of linkage of bacterial proteins to host polysaccharide receptors and alkaloids complexes make their effect by inhibiting of efflux pump ,

Finally, Anticandidiasis efficacy of (*D. caryophyllus* L.) might be belonging to phytochemical compounds such as terpenoids, flavonoids, Alkaloids and their effect in proteins and polysaccharides and disruption in membranes permeability or inhibiting of efflux pump or DNA synthesis.

4.3.2. Antifungal activity of *Saussurea costus*

The results of antifungal activity of the crude Alkaloid compounds extracted from the roots of (*Saussurea costus*) against *Candida* species isolated from different clinical samples such as mouth and vagina are presented in table (4-10). The antifungal activity of Alkaloid secondary metabolites with three concentrations (25, 50, and 100mg/ml) was screened by agar well diffusion methods.

The results revealed that, the crude Alkaloid compounds extracted from the roots of (*Saussurea costus*) showed significant reduction at $P \leq 0.05$ in the growth of *Candida* species. Growth inhibition represented by zone of inhibition ranging from $(20 \pm 1.00\text{mm})$ in 25 mg/ml, 25 ± 1.00 mm in 50 mg/ ml, and 30 ± 1.00 mm in 100 mg/ml) (Figure: 1 in 4-12), compared with negative control representative by 10% DMSO and positive control representative by Fluconazole 50mg/ml where inhibition zone was (0.00 mm for negative control and $35 \pm 1.00\text{mm}$ for positive control).

On the other hand, the crude Flavonoid compounds showed $8 \pm 1.00\text{mm}$ of zone of inhibition at (25 mg/ml) and $12 \pm 1.00\text{mm}$ at (50 mg/ml), and $27 \pm 1.00\text{mm}$ at (100 mg/ml) concentration (table 4-11),

Thus, it differed significantly compared to the control treatment (Figure: 2 in 4-12).

In the same context, the crude Terpenoid compounds showed significant activity at three concentrations (25, 50, and 100 mg/ml) compared with negative control against *Candida* species isolated from different clinical samples Table (4-12). The highest zone of inhibition 31 ± 1.00 mm was recorded at 100 mg/ml and 26 ± 1.00 mm was recorded at 50 mg/ml (figure: 3 in 4-12).

While the highest zone of inhibition in the crude Alkaloid compounds was reached up to 30 ± 1.00 mm at 100 mg/ml concentration and the highest zone of inhibition in the crude Flavonoid compounds was reached up to 27 ± 1.00 at 100 mg/ml concentration. The terpenoid and alkaloid compounds were the most effective compared to flavonoid compounds and came close to the effect of the Fluconazole (35 ± 1.00 mm), figure (4-13).

Table (4-10) Anti-candidiasis activity of the crude Alkaloid compounds extracted from *Saussurea costus* roots

| Concentrations (mg/ml) | Alkaloids compounds |
|--------------------------------|---------------------|
| | Inhibition Zone/mm |
| Negative Control | 0 ± 0.00 |
| 25 mg/ml | 20 ± 1.00 |
| 50 mg/ml | 25 ± 1.00 |
| 100 mg/ml | 30 ± 1.00 |
| Positive Control | 35 ± 1.00 |
| L.S. D | 1.62 |
| *Mean \pm standard deviation | |

Table (4-11) Anti-candidiasis activity of the crude Flavonoid compounds extracted from *Saussurea costus* roots

| Concentrations (mg/ml) | Flavonoid compounds |
|---------------------------|---------------------|
| | Inhibition zone % |
| Negative Control | 0± 0.00 |
| 25 mg/ml | 8± 1.00 |
| 50 mg/ml | 12± 1.00 |
| 100 mg/ml | 27± 1.00 |
| Positive Control | 35± 1.00 |
| L.S. D | 1.00 |
| *Mean± standard deviation | |

Table (4-12) Anti-candidiasis activity of the crude Terpenoid compounds extracted from *Saussurea costus* roots

| Concentrations (mg/ml) | Terpenoid compounds |
|---------------------------|---------------------|
| | Inhibition zone % |
| Negative Control | 0± 0.00 |
| 25 mg/ml | 22± 1.00 |
| 50 mg/ml | 26± 1.00 |
| 100 mg/ml | 31± 1.00 |
| Positive Control | 35± 1.00 |
| L.S. D | 0.57 |
| *Mean± standard deviation | |

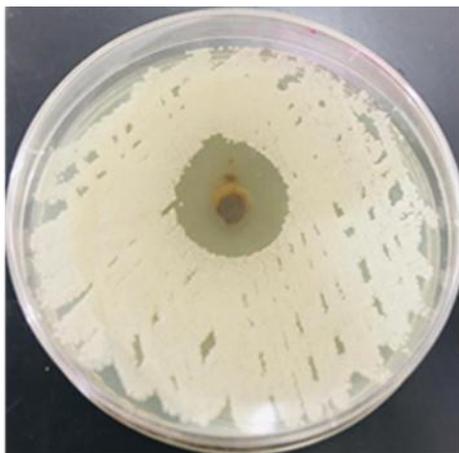


Figure 1: Anti-candidiasis activity of the crude Alkaloid compounds extracted from *Saussurea costus* roots at 100 mg/ml



Figure 2: Anti-candidiasis activity of the crude Flavonoid compounds extracted from *Saussurea costus* roots at 100 mg/ml

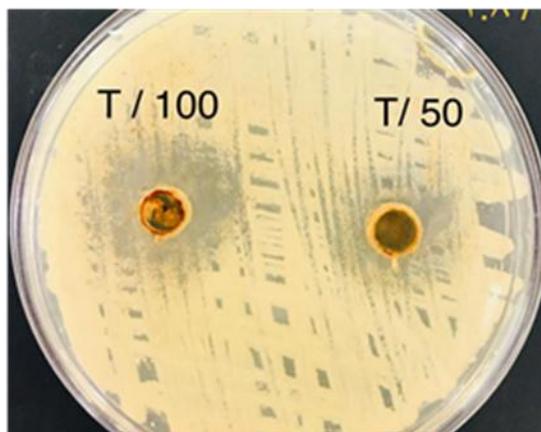


Figure 3: Anti-candidiasis activity of the crude Flavonoid compounds extracted from *Saussurea costus* roots at 50 and 100 mg/ml

Figure (4-13) Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at

100/ml against of *Saussurea costus* roots *Candida* species, LSD= 1.82

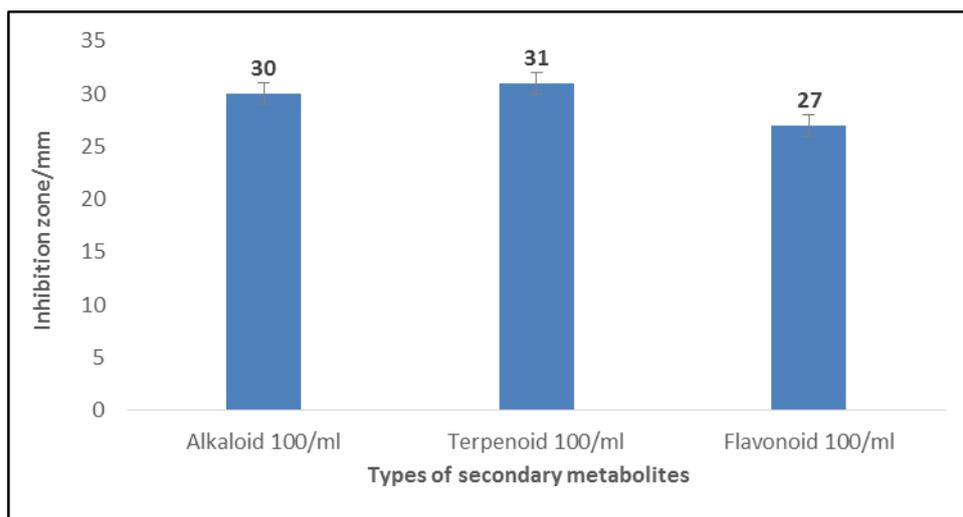


Figure (4-14) Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml against *Candida* species, LSD= 2.15

The present study was proved that, the secondary metabolites include Alkaloids, Flavonoids, and Terpenoids extracted from the roots of (*Saussurea costus*) had antifungal activity against *Candida* species isolated from different clinical samples such as mouth and vagina. The plant kingdom provided and is still providing endless sources of medicinal plants of various uses for example,

Secondary metabolites extracted from different active parts of numerous medicinal plants such as (*Lactuca serriola* leaves; *Lepidium sativum* leaves; *Myrtus Communis* leaves; *Cassia senna* leaves; *Ricinus communis* leaves; *Cassia didymobotrya* leaves; *Melia azedarach* leaves; *Dianthus caryophyllus* flowers bud; and *Salvia hispanica* seeds),

Possess ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples Reported that, phytochemical compounds extracted from the unicellular primitive plant like *Chlorella vulgaris* possess ability of antibacterial counter to pathogenic bacteria Used phytochemical compounds extracted from *Hibiscus sabdarifa* for controlling *E. coli* and *Proteus* sp Used

phytochemical compounds extracted from of *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa* (Hussein,*et al.*, 2020). Used phytochemical compounds extracted from *Boswellia carteri* and *Curcuma longa* for controlling *Fusarium* sp. isolated from seeds of maize. (Hussein,*et al.*, 2020).

Used terpenoids compounds extracted from *C. tinctorius* seeds and flavonoid compounds extracted from *M. Communis* leaves against *Aspergillus* species isolated from stored medicinal plant seeds. Secondary metabolites represented by Alkaloids and Flavonoids compounds extracted from *M. Communis* leaves respected a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens (Radhi,*et al.*,2020).

Used *Callistemon viminalis* leaves extracts for controlling isolates of Urinary Tract Infections. *S. costus* has been screened as medicinally important plant, the various chemical compounds isolated from it possess medicinal properties (Kamalpreet,*e al*, 2019).

Various compounds were isolated from the roots of *S. costus* and tested against the nine fungal strains *i.e.* *Aspergillus flavus*, *Aspergillusniger*, *Aspergillus ochraceus*, *Aspergillus versicolor*, *Aspergillus flavus*, *Penicilium ochrochloron*, *Penicilium funiculosum*, *Trichodermaviride*, *Cladosporium cladosporioides* and *Alternaria*. The compound showed antifungal effects which were moderate too high (Rao,*etal.*,2007).

Ethanol and ethyl acetate extracts of *S. costus* had the highest levels of polyphenols followed by n-butanol, and then n-hexane extracts. The main phenolic compounds are Naringenin, Chlorogenic acid, Ferulic acid, Ellagic acid, Gallic acid and coffeic acid followed by taxifolin, catechin,

syringic acid, methyl gallate, vanillin, kaempferol, cinnamic acid and rutin and this extracts had antibacterial and antifungal agents against broad range of microorganisms (*Saussurea lappa* showed best antifungal activity against *Aspergillus flavus* followed by *Trapa natans* and *Mangifera indica* (Parekh, & Chanda ,2008). The methanol extract of roots of *S. costus* are rich in some bioactive phytochemical compounds such as alkaloids, phenols/polyphenols, flavonoids, terpenoids, tannins, coumarins, quinines, steroids, cardiac glycosides and resins and had antifungal activity against *Aspergillus niger* ATCC 6275 (Abdallah,*etal.*,2017).

The crude extracts of n-hexane and dichloromethane were effective against different strains and isolates of *Candida* (Soliman,*etal.*,2022).

On the other hands, the mode of the antifungal action of the Alkaloids is usually pleiotropic, where protein synthesis is inhibited, and the fungal DNA is intercalated or by boosting the development of fungi inhibitors (Parekh, & Chanda ,2008).

Terpenoids reduced the mitochondrial content, thus modified the level of reactive oxygen species (ROS) and ATP generation. It is also reported that triterpenoid possesses more potent antifungal activity as compared to the tetraterpenoid (Abdallah*etal.*, 2017).

Terpenoids and flavonoids make their effects by disruption of microbial membranes (Soliman *etal.*, 2022). Medicinal plant possessed antifungal effects by many mechanisms, they caused membrane disturbance resulting in the loss of membrane integrity, inhibited DNA transcription and reduced the cell populations, inhibited the activity of fungal antioxidant enzymes and inhibited fungal biofilm formation (Arif *etal.*, 2009).

Finally, anti-candidiasis activity of *Carthamus tinctorius* L. seeds might be belonging to secondary metabolites like Alkaloids, Flavonoids, and Terpenoids and their effect in proteins and DNA synthesis and disruption in membranes permeability or disturbance in metabolic activity.

4.3.3. Antifungal activity of *Carthamus tinctorius* L. Seeds result

The results of antifungal activity of the crude Alkaloid compounds extracted from the seeds of (*Carthamus tinctorius* L.) against *Candida* species isolated from different clinical samples such as mouth and vagina are presented in (table 4-12).

The antifungal activity of Alkaloid secondary metabolites with three concentration (25, 50, and 100mg/ml) was screened by agar well diffusion methods. The results revealed that, the crude Alkaloid compounds extracted from the seeds of (*Carthamus tinctorius* L.) showed significant reduction at $P \leq 0.05$ in the growth of *Candida* species. Growth inhibition represented by zone of inhibition ranging from $(22 \pm 1.00$ mm in 25 mg/ml, 26 ± 1.00 mm in 50 mg/ml, and 30 ± 1.00 mm in 100 mg/ml) (Figure: 1 in 4-14), compared with negative control representative by 10% DMSO and positive control representative by Fluconazole 50mg/ml (Figure: 2 in 4-14) where inhibition zone was (0.00 mm for negative control and 35 ± 1.00 mm for positive control). On the other hand, the crude Flavonoid compounds showed 8 ± 1.00 mm of zone of inhibition at (25 mg/ml) and 12 ± 1.00 mm at (50 mg/ml), and 21 ± 1.00 mm at (100 mg/ml) concentration (table 4-14), Thus, it differed significantly compared to the control treatment (Figure: 3 in 4-14).

In the same context, the crude Terpenoid compounds showed significant activity at three concentrations (25, 50, and 100 mg/ml)

compared with negative control against *Candida* species isolated from different clinical samples (Table 4-15). The highest zone of inhibition 26 ± 1.00 mm was recorded at 100 mg/ml and 24 ± 1.00 mm was recorded at 50 mg/ml (figure: 4).

While the highest zone of inhibition in the crude Alkaloid compounds was reached up to 30 ± 1.00 mm at 100 mg/ml concentration and the highest zone of inhibition in the crude Flavonoid compounds was reached up to 21 ± 1.00 at 100 mg/ml concentration (Figure: 4-15). The alkaloid compounds were the most effective compared to the rest of the active compounds such as Terpenoids and Flavonoid came close to the effect of the Fluconazole (35 ± 1.00 mm).

Table (4-13) Anti-candidiasis activity of the crude Alkaloid compounds extracted from (*Carthamus tinctorius* L) seeds

| Concentrations (mg/ml) | Alkaloids compounds |
|--|---------------------|
| | Inhibition Zone/mm |
| Negative Control | 0 ± 0.00 |
| 25 mg/ml | 22 ± 1.00 |
| 50 mg/ml | 26 ± 1.00 |
| 100 mg/ml | 30 ± 1.00 |
| Positive Control | 35 ± 1.00 |
| L.S. D | 1.62 |
| *Mean \pm standard deviation | |

Table (4-14) Anti-candidiasis activity of the crude Flavonoid compounds extracted from (*Carthamus tinctorius* L.) seeds

| Concentrations (mg/ml) | Flavonoid compounds |
|-------------------------|---------------------|
| | Inhibition zone % |
| Negative Control | 0 ± 0.00 |
| 25 mg/ml | 8 ± 1.00 |

| | |
|----------------------------------|----------|
| 50 mg/ml | 12± 1.00 |
| 100 mg/ml | 21± 1.00 |
| Positive Control | 35± 1.00 |
| L.S. D | 0.33 |
| *Mean± standard deviation | |

Table (4-15) Anti-candidiasis activity of the crude Terpenoid compounds extracted from (*Carthamus tinctorius L.*) seeds

| Concentrations (mg/ml) | Terpenoid compounds |
|----------------------------------|---------------------|
| | Inhibition zone % |
| Negative Control | 0± 0.00 |
| 25 mg/ml | 21± 1.00 |
| 50 mg/ml | 24± 1.00 |
| 100 mg/ml | 26± 1.00 |
| Positive Control | 35± 1.00 |
| L.S. D | 1.00 |
| *Mean± standard deviation | |

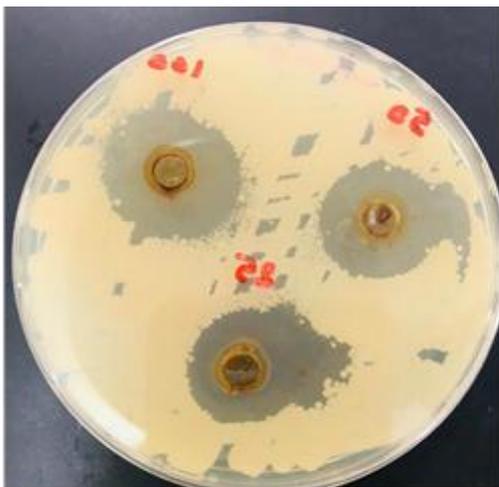


Figure 1: Anti-candidiasis activity of the crude Alkaloid compounds extracted from (*C. tinctorius* L) seeds at 25, 50, and 100 mg/ml



Figure 2: Fluconazole as a positive control at 50mg/ml

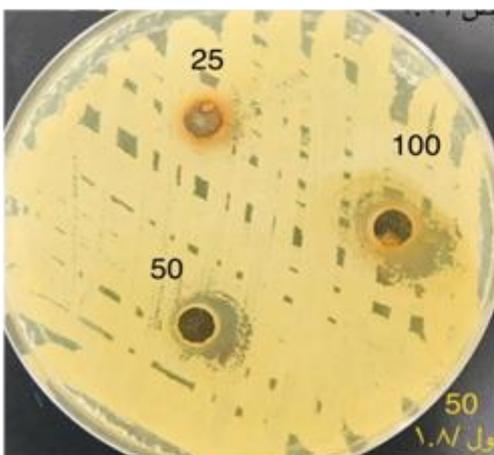


Figure 3: Anti-candidiasis activity of the crude Flavonoid compounds extracted from (*C. tinctorius* L.) seeds at 25, 50, and 100 mg/ml

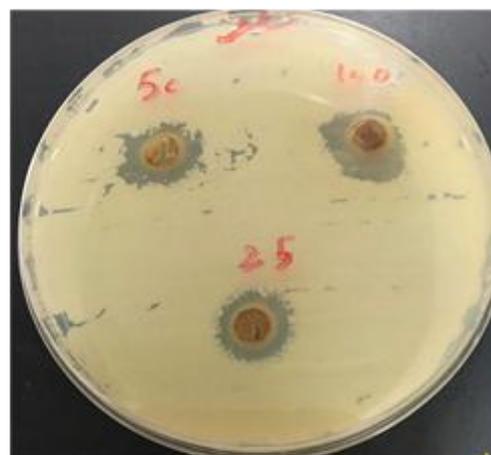


Figure 4: Anti-candidiasis activity of the crude Terpenoid compounds extracted from (*C. tinctorius* L.) seeds at 25, 50, and 100 mg/ml

Fig (4-15) Anti-candidiasis activity of the crude Terpenoid compounds extracted from (*Carthamus tinctorius* L.) seeds

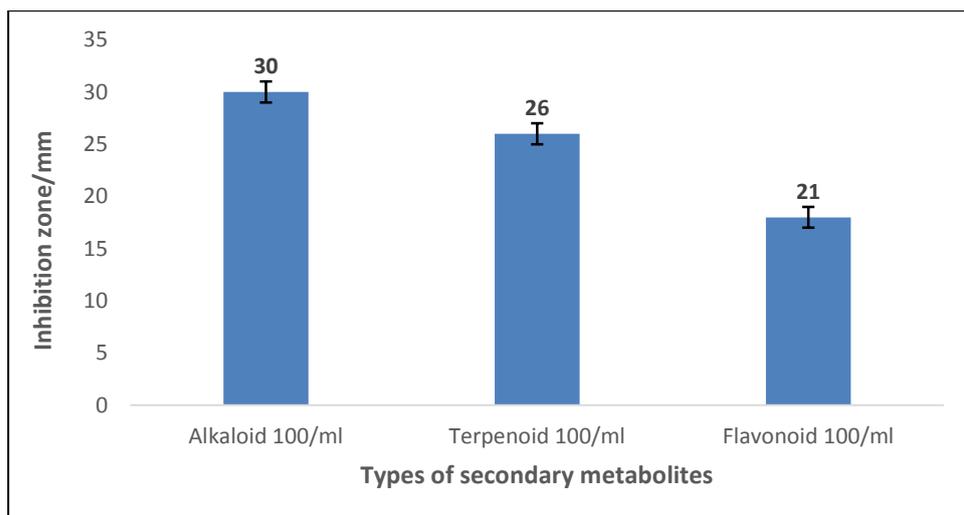


Figure (4-16): Inhibition zone of Alkaloid, Terpenoid, and Flavonoid at 100/ml against *Candida* species, LSD= 1.81

The present study was proved that, the secondary metabolites include Alkaloids, Flavonoids, and Terpenoids extracted from the seeds of (*Carthamus tinctorius* L.) have powerful antifungal activity against *Candida* species isolated from different clinical samples such as mouth and vagina. The plant kingdom provided and is still providing endless sources of medicinal plants of various uses for example, Secondary metabolites extracted from different active parts of numerous medicinal plants such as (*Lactuca serriola* leaves; *Lepidium sativum* leaves; *Myrtus Communis* leaves; *Cassia senna* leaves; *Ricinus communis* leaves; *Cassia didymobotrya* leaves; *Melia azedarach* leaves; *Dianthus caryophyllus* flowers bud; and *Salvia hispanica* seeds), possess ability of antibacterials for controlling several pathogenic microorganisms isolated from different clinical samples (Hussein, & Al-Marzoqi,2020). Reported that, phytochemical compounds extracted from the unicellular primitive plant like *Chlorella vulgaris* possess ability of antibacterial counter to pathogenic bacteria used phytochemical compounds extracted from *Hibiscus sabdarifa* for controlling *E. coli* and *Proteus*

sp(Kamal,*etal*,.2020).Used phytochemical compounds extracted from of *Ficus carica* L. for controlling *E. coli* and *Pseudomonas aeruginosa* (AL-Masoodi *etal*, 2020). Used phytochemical compounds extracted from *Boswellia carteri* and *Curcuma longa* for controlling *Fusarium* sp. isolated from seeds of maize(Hussain *etal*, 2021).Used terpenoids compounds extracted from *C. tinctorius* seeds and flavonoid compounds extracted from *M. Communis* leaves against *Aspergillus* species isolated from stored medicinal plant seeds. Secondary metabolites represented by Alkaloids and Flavonoids compounds extracted from *M. Communis* leaves respected a worthy source for controlling pathogenic microorganisms segregated from hemodialysis fluid specimens (Sharara *etal*, 2021). Used *Callistemon viminalis* leaves extracts for controlling isolates of Urinary Tract Infections. The antifungal activity of the carthamin natural pigment of safflower was more active against *Candida albicans* than precarthamin (Salem *etal*, 2014). Safflower oil extracted from seeds exhibited a significant antifungal activity against *Candida parapsilosis* and *Candida sake* (Khémiri *etal*, 2020).On the other hands, the mode of the antifungal action of the Alkaloids is usually pleiotropic, where protein synthesis is inhibited, and the fungal DNA is intercalated or by boosting the development of fungi inhibitors (Arif,et al, 2009). Terpenoids reduced the mitochondrial content, thus modified the level of reactive oxygen species (ROS) and ATP generation. It is also reported that triterpenoid possesses more potent antifungal activity as compared to the tetraterpenoid (Haque *et al*, 2016). Terpenoids and flavonoids make their effects by disruption of microbial membranes (Okusa *etal*, 2009). Medicinal plant possessed antifungal effects by many mechanisms, they caused membrane disturbance resulting in the loss of membrane integrity, inhibited DNA transcription and reduced the cell populations, inhibited the activity of fungal antioxidant enzymes and inhibited fungal biofilm

formation (Wu *etal*, 2013). Finally, anti-candidiasis activity of *Carthamus tinctorius* L. seeds might be belonging to secondary metabolites like Alkaloids, Flavonoids, and Terpenoids and their effect in proteins and DNA synthesis and disruption in membranes permeability or disturbance in metabolic activity.

Chapter Five

Conclusions & Recommendations

5.1 Conclusion

1. Secondary metabolites compounds extracted from flower buds *D. caryophyllus* L. such as terpenoid and flavonoid regard a good source for controlling *Candida* species isolated from different clinical samples.
2. Alkaloids, and Terpenoids extracted from the seeds of (*Saussurea costus*) have powerful antifungal activity against *Candida* species.
3. Alkaloids, Flavonoids, and Terpenoids extracted from the seeds of (*Carthamus tinctorius* L.) have powerful antifungal activity against *Candida* species.
4. *Dianthus caryophyllus* L the best in antagonistic activity when focusing (25 , 50 , 100) in Alkaloids, Flavonoids, and Terpenoids

5.2 Recommendations

In order to complete the study and research on all aspects related to fungi and yeast we recommend the following .:

Continuing to conduct studies on the pathology of other fungi that cause candidiasis .

References

References

- Buggio, L., Somigliana, E., Borghi, A., & Vercellini, P. (2019). Probiotics and vaginal microecology: fact or fancy?. *BMC women's health*, 19(1), 1-6.
- Diao, K., Han, P., Pang, T., Li, Y., & Yang, Z. (2020). HRCT imaging features in representative imported cases of 2019 novel coronavirus pneumonia. *Precision Clinical Medicine*, 3(1), 9-13.
- Rocha, M. F. G., Sales, J. A., da Rocha, M. G., Galdino, L. M., de Aguiar, L., Pereira-Neto, W. D. A., ... & Brilhante, R. S. N. (2019). Antifungal effects of the flavonoids kaempferol and quercetin: A possible alternative for the control of fungal biofilms. *Biofouling*, 35(3), 320-328.
- Swor, K., Satyal, P., Timsina, S., & Setzer, W. N. (2022). Chemical Composition and Terpenoid Enantiomeric Distribution of the Essential oil of *Artemisia tridentata* Subsp. *tridentata* From Southwestern Idaho. *Natural Product Communications*, 17(7), 1934578X221117417.
- Wilson, D. (2019). *Candida albicans*. *Trends in Microbiology*, 27(2), 188-189.
- Wu, S. C., Liu, F., Zhu, K., & Shen, J. Z. (2019). Natural products that target virulence factors in antibiotic-resistant *Staphylococcus aureus*. *Journal of agricultural and food chemistry*, 67(48), 13195-13211.
- Abdallah, E. M., Qureshi, K. A., Ali, A. M., & Elhassan, G. O. (2017). Evaluation of some biological properties of *Saussurea costus* crude root extract. *Biosci. Biotechnol. Res. Commun*, 10(4), 601-611.
- Abdul, R. B. (2012). Medicinal plants (Importants and uses). *Pharm. Anal. Acta*, 3, e139.
- Agati, G., Brunetti, C., Fini, A., Gori, A., Guidi, L., Landi, M., ... & Tattini, M. (2020). Are flavonoids effective antioxidants in plants? Twenty years of our investigation. *Antioxidants*, 9(11), 1098.
- Aguirre-Quñonero, A., Castillo-Sedano, I., Calvo-Muro, F., & Canut-Blasco, A. (2019). Accuracy of the BD MAX™ vaginal panel in the diagnosis of infectious vaginitis. *European Journal of Clinical Microbiology & Infectious Diseases*, 38(5), 877-882.
- Ahn, K. (2017). The worldwide trend of using botanical drugs and strategies for developing global drugs. *BMB reports*, 50(3), 111.
- Ajikumar, P. K., Tyo, K., Carlsen, S., Mucha, O., Phon, T. H., & Stephanopoulos, G. (2008). Terpenoids: opportunities for biosynthesis of

References

- natural product drugs using engineered microorganisms. *Molecular pharmaceuticals*, 5(2), 167-190.
- Algammal, A. M., Hetta, H. F., Elkelish, A., Alkhalifah, D. H. H., Hozzein, W. N., Batiha, G. E. S., ... & Mabrok, M. A. (2020). Methicillin-Resistant *Staphylococcus aureus* (MRSA): one health perspective approach to the bacterium epidemiology, virulence factors, antibiotic-resistance, and zoonotic impact. *Infection and Drug Resistance*, 13, 3255.
 - Alhasani, A. H., Al-Akwa, A. A. Y., Al-Shamahy, H. A., Al-deen, H. M. S., & Al-labani, M. A. (2020). Biofilm formation and antifungal susceptibility of candida isolates from oral cavity after the introduction of fixed orthodontic appliances. *Univ J Pharm Res*, 5, 21-27.
 - AL-Masoodi, H., Hussein, H. J., & Al-Rubaye, A. F. (2020). Antifungal activity of the two medicinal plants (*Curcuma longa* L. and *Boswellia carteri* Birdwood) against *Fusarium* species isolated from maize seeds. *International Journal of Pharmaceutical Research*, 12(3), 408-414.
 - Almirante, B., Rodríguez, D., Cuenca-Estrella, M., Almela, M., Sanchez, F., Ayats, J., ... & Barcelona Candidemia Project Study Group. (2006). Epidemiology, risk factors, and prognosis of *Candida parapsilosis* bloodstream infections: case-control population-based surveillance study of patients in Barcelona, Spain, from 2002 to 2003. *Journal of clinical microbiology*, 44(5), 1681-1685.
 - Al-Snafi, A. E. (2017). Chemical contents and medical importance of *Dianthus caryophyllus*-A review. *IOSR Journal of Pharmacy*, 7(3), 61-71.
 - Ambavade, S. D., Mhetre, N. A., Muthal, A. P., & Bodhankar, S. L. (2009). Pharmacological evaluation of anticonvulsant activity of root extract of *Saussurea lappa* in mice. *European Journal of Integrative Medicine*, 1(3), 131-137.
 - Amsel, R., Totten, P. A., Spiegel, C. A., Chen, K. C., Eschenbach, D., & Holmes, K. K. (1983). Nonspecific vaginitis: diagnostic criteria and microbial and epidemiologic associations. *The American journal of medicine*, 74(1), 14-22.
 - Anand, U., Jacobo-Herrera, N., Altemimi, A., & Lakhssassi, N. (2019). A comprehensive review on medicinal plants as antimicrobial therapeutics: potential avenues of biocompatible drug discovery. *Metabolites*, 9(11), 258.

References

- Andersen, O. M., & Markham, K. R. (2005). Flavonoids: chemistry, biochemistry and applications. CRC press.
- Andersen, O. M., & Markham, K. R. (2005). Flavonoids: chemistry, biochemistry and applications. CRC press.
- Andes, D. R., Safdar, N., Baddley, J. W., Playford, G., Reboli, A. C., Rex, J. H., ... & Kullberg, B. J. (2012). Impact of treatment strategy on outcomes in patients with candidemia and other forms of invasive candidiasis: a patient-level quantitative review of randomized trials. *Clinical infectious diseases*, 54(8), 1110-1122.
- Andrew, N. H., Ruberu, R. P., & Gabb, G. (2011). The first documented case of *Candida dubliniensis* leptomenigeal disease in an immunocompetent host. *Case Reports*, 2011, bcr0620114384.
- Ang-Lee, M. K., Moss, J., & Yuan, C. S. (2001). Herbal medicines and perioperative care. *Jama*, 286(2), 208-216.
- Antinori, S., Milazzo, L., Sollima, S., Galli, M., & Corbellino, M. (2016). Candidemia and invasive candidiasis in adults: A narrative review. *European journal of internal medicine*, 34, 21-28.
- Arendrup, M. C., & Perlin, D. S. (2014). Echinocandin resistance: an emerging clinical problem?. *Current opinion in infectious diseases*, 27(6), 484.
- Arif, T., Bhosale, J. D., Kumar, N., Mandal, T. K., Bendre, R. S., Lavekar, G. S., & Dabur, R. (2009). Natural products–antifungal agents derived from plants. *Journal of Asian natural products research*, 11(7), 621-638.
- Arif, T., Bhosale, J. D., Kumar, N., Mandal, T. K., Bendre, R. S., Lavekar, G. S., & Dabur, R. (2009). Natural products–antifungal agents derived from plants. *Journal of Asian natural products research*, 11(7), 621-638.
- Arimura, G. I., Ozawa, R., Shimoda, T., Nishioka, T., Boland, W., & Takabayashi, J. (2000). Herbivory-induced volatiles elicit defence genes in lima bean leaves. *Nature*, 406(6795), 512-515.
- Asgarpanah, J., & Kazemivash, N. (2013). Phytochemistry, pharmacology and medicinal properties of *Carthamus tinctorius* L. *Chinese journal of integrative medicine*, 19(2), 153-159.
- Asgary, S., Rahimi, P., Mahzouni, P., & Madani, H. (2012). Antidiabetic effect of hydroalcoholic extract of *Carthamus tinctorius* L. in alloxan-induced

References

- diabetic rats. *Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences*, 17(4), 386.
- Atarod, M., Safari, J., & Tebyanian, H. (2020). Ultrasound irradiation and green synthesized CuO-NiO-ZnO mixed metal oxide: An efficient sono/nano-catalytic system toward a regioselective synthesis of 1-aryl-5-amino-1 H-tetrazoles. *Synthetic Communications*, 50(13), 1993-2006.
 - Azie, N., Angulo, D., Dehn, B., & Sobel, J. D. (2020). Oral Ibrexafungerp: an investigational agent for the treatment of vulvovaginal candidiasis. *Expert Opinion on Investigational Drugs*, 29(9), 893-900.
 - Bae, C. S., Park, C. H., Cho, H. J., Han, H. J., Kang, S. S., Choi, S. H., & Uhm, C. S. (2002). Therapeutic effects of safflower (*Carthamus tinctorius* L.) seed powder on osteoporosis. *Applied Microscopy*, 32(3), 285-290.
 - Basso, V., d'Enfert, C., Znaidi, S., & Bachellier-Bassi, S. (2018). From genes to networks: the regulatory circuitry controlling *Candida albicans* morphogenesis. *Fungal Physiology and Immunopathogenesis*, 61-99.
 - Becker, H. (1996). Jean Bruneton, *Pharmacognosy, Phytochemistry, Medicinal Plants (1995)*, Intercept, pp. 915.(ISBN 1-898298-13-0). Price: 130.00 Pound Sterling.
 - Becker, H. (1996). Jean Bruneton, *Pharmacognosy, Phytochemistry, Medicinal Plants (1995)*, Intercept, pp. 915.(ISBN 1-898298-13-0). Price: 130.00 Pound Sterling.
 - Bednarz, H., Roloff, N., & Niehaus, K. (2019). Mass spectrometry imaging of the spatial and temporal localization of alkaloids in Nightshades. *Journal of agricultural and food chemistry*, 67(49), 13470-13477.
 - Beigi, R. H., Meyn, L. A., Moore, D. M., Krohn, M. A., & Hillier, S. L. (2004). Vaginal yeast colonization in nonpregnant women: a longitudinal study. *Obstetrics & Gynecology*, 104(5), 926-930.
 - Berg, A. O., Heidrich, F. E., Fihn, S. D., Bergman, J. J., Wood, R. W., Stamm, W. E., & Holmes, K. K. (1984). Establishing the cause of genitourinary symptoms in women in a family practice: comparison of clinical examination and comprehensive microbiology. *Jama*, 251(5), 620-625

References

- Bersani, I., Piersigilli, F., Goffredo, B. M., Santisi, A., Cairoli, S., Ronchetti, M. P., & Auriti, C. (2019). Antifungal drugs for invasive candida infections (ICI) in neonates: Future perspectives. *Frontiers in pediatrics*, 7, 375.
- Berville, A., Breton, C., Cunliffe, K., Darmency, H., Good, A. G., Gressel, J., ... & Warwick, S. I. (2005). Issues of ferality or potential for ferality in oats, olives, the Vigna group, ryegrass species, safflower, and sugarcane. *Crop ferality and volunteerism*, 231-255.
- Borghi, E., Borgo, F., & Morace, G. (2016). Fungal biofilms: update on resistance. *Fungal Biofilms and related infections*, 37-47.
- Breitmaier, E. (2006). Terpenes: Importance, general structure, and biosynthesis. *Terpenes: Flavors, fragrances, pharmaca, pheromones*, 1, 1-3.
- Bribi, N. (2018). Pharmacological activity of alkaloids: a review. *Asian Journal of Botany*, 1(1), 1-6.
- Brodie, C., Tordai, A., Saloga, J., Domenico, J., & Gelfand, E. W. (1995). Ouabain induces inhibition of the progression phase in human T-cell proliferation. *Journal of cellular physiology*, 165(2), 246-253.
- Brotman, R. M., Klebanoff, M. A., Nansel, T. R., Yu, K. F., Andrews, W. W., Zhang, J., & Schwebke, J. R. (2010). Bacterial vaginosis assessed by gram stain and diminished colonization resistance to incident gonococcal, chlamydial, and trichomonal genital infection. *Journal of Infectious Diseases*, 202(12), 1907-1915.
- Butler, G., Rasmussen, M. D., Lin, M. F., Santos, M. A., Sakthikumar, S., Munro, C. A., ... & Cuomo, C. A. (2009). Evolution of pathogenicity and sexual reproduction in eight *Candida* genomes. *Nature*, 459(7247), 657-662.
- Camargo, K. C. D., Alves, R. R. F., Baylão, L. A., Ribeiro, A. A., Araujo, N. L. A. D. S., Tavares, S. B. D. N., & Santos, S. H. R. D. (2015). Secreção vaginal anormal: Sensibilidade, especificidade e concordância entre o diagnóstico clínico e citológico. *Revista Brasileira de Ginecologia e Obstetrícia*, 37, 222-228.
- Camargo, K. C. D., Alves, R. R. F., Baylão, L. A., Ribeiro, A. A., Araujo, N. L. A. D. S., Tavares, S. B. D. N., & Santos, S. H. R. D. (2015). Secreção vaginal anormal: Sensibilidade, especificidade e concordância entre o diagnóstico clínico e citológico. *Revista Brasileira de Ginecologia e Obstetrícia*, 37, 222-228.

References

- Castanheira, M., Messer, S. A., Rhomberg, P. R., & Pfaller, M. A. (2016). Antifungal susceptibility patterns of a global collection of fungal isolates: results of the SENTRY Antifungal Surveillance Program (2013). *Diagnostic microbiology and infectious disease*, 85(2), 200-204.
- Castellani, A. (1912). ii. Observations on some Intestinal Bacteria found in Man. *Zentralblatt fur Bakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene*, 65(4-5).
- Chandra S, Rawat DS, Chandra D and Rastogi J. Nativity, phytochemistry, ethnobotany and pharmacology of *Dianthus caryophyllus*. *Research Journal of Medicinal Plant*. 2016; 10 (1): 1-9.
- Chandra, S., Rawat, D. S., Chandra, D., & Rastogi, J. (2016). Nativity, phytochemistry, ethnobotany and pharmacology of *Dianthus caryophyllus*. *Research Journal of Medicinal Plant*, 10(1), 1-9.
- Chavan, S. S., Damale, M. G., Devanand, B., & Sangshetti, J. N. (2018). Antibacterial and antifungal drugs from natural source: A review of clinical development. *Natural Products in Clinical Trials: Sharjah, UAE*, 1, 114.
- Cho, I., Jackson, M. R., & Swift, J. (2016). Roles of cross-membrane transport and signaling in the maintenance of cellular homeostasis. *Cellular and Molecular Bioengineering*, 9(2), 234-246.
- Cho, J. Y., Baik, K. U., Jung, J. H., & Park, M. H. (2000). In vitro anti-inflammatory effects of cynaropicrin, a sesquiterpene lactone, from *Saussurea lappa*. *European Journal of Pharmacology*, 398(3), 399-407.
- Choi, J., & Kim, S. H. (2017). A genome tree of life for the fungi kingdom. *Proceedings of the National Academy of Sciences*, 114(35), 9391-9396.
- Chowdhary, A., Sharma, C., & Meis, J. F. (2017). *Candida auris*: a rapidly emerging cause of hospital-acquired multidrug-resistant fungal infections globally. *PLOS pathogens*, 13(5), e1006290.
- Ciurea, C. N., Kosovski, I. B., Mare, A. D., Toma, F., Pintea-Simon, I. A., & Man, A. (2020). *Candida* and candidiasis—opportunism versus pathogenicity: a review of the virulence traits. *Microorganisms*, 8(6), 857.
- Clancy, C. J., & Nguyen, M. H. (2017). Emergence of *Candida auris*: an international call to arms. *Clinical Infectious Diseases*, 64(2), 141-143.
- Clancy, C. J., & Nguyen, M. H. (2018). Diagnosing invasive candidiasis. *Journal of clinical microbiology*, 56(5), e01909-17.

References

- Cleveland, A. A., Harrison, L. H., Farley, M. M., Hollick, R., Stein, B., Chiller, T. M., ... & Park, B. J. (2015). Declining incidence of candidemia and the shifting epidemiology of *Candida* resistance in two US metropolitan areas, 2008–2013: results from population-based surveillance. *PloS one*, 10(3), e0120452.
- Colombo, A. L., Guimarães, T., Silva, L. R., de Almeida Monfardini, L. P., Cunha, A. K. B., Rady, P., ... & Rosas, R. C. (2007). Prospective observational study of candidemia in Sao Paulo, Brazil: incidence rate, epidemiology, and predictors of mortality. *Infection Control & Hospital Epidemiology*, 28(5), 570-576.
- Cui, J., Ren, B., Tong, Y., Dai, H., & Zhang, L. (2015). Synergistic combinations of antifungals and anti-virulence agents to fight against *Candida albicans*. *Virulence*, 6(4), 362-371.
- Dangi, Y. S., Soni, M. L., & Namdeo, K. P. (2010). Oral candidiasis: A review. *Int J Pharm Pharm Sci*, 2(4), 36-41.
- Das, S. N., Patro, V. J., & Dinda, S. C. (2012). A review: Ethnobotanical survey of genus *Leucas*. *Pharmacognosy reviews*, 6(12), 100.
- de Barros, P. P., Rossoni, R. D., Freire, F., Ribeiro, F. D. C., Lopes, L. A. D. C., Junqueira, J. C., & Jorge, A. O. C. (2018). *Candida tropicalis* affects the virulence profile of *Candida albicans*: an in vitro and in vivo study. *Pathogens and disease*, 76(2), fty014.
- de Paula Menezes, R., Silva, F. F., Melo, S. G., Alves, P. G., Brito, M. O., de Souza Bessa, M. A., ... & Röder, D. V. D. (2019). Characterization of *Candida* species isolated from the hands of the healthcare workers in the neonatal intensive care unit. *Medical mycology*, 57(5), 588-594.
- Deabes, M. M., Fatah, A. E., Sally, I., Salem, S. H. E., & Naguib, K. M. (2021). Antimicrobial activity of bioactive compounds extract from *Saussurea costus* against food spoilage microorganisms. *Egyptian Journal of Chemistry*, 64(6), 2833-2843.
- Delshad, E., Yousefi, M., Sasannezhad, P., Rakhshandeh, H., & Ayati, Z. (2018). Medical uses of *Carthamus tinctorius* L.(safflower): a comprehensive review from traditional medicine to modern medicine. *Electronic physician*, 10(4), 6672.

References

- Demuyser, L., Jabra-Rizk, M. A., & Van Dijck, P. (2014). Microbial cell surface proteins and secreted metabolites involved in multispecies biofilms. *Pathogens and disease*, 70(3), 219-230.
- Deng, K., Jiang, W., Jiang, Y., Deng, Q., Cao, J., Yang, W., & Zhao, X. (2021). ALS3 expression as an indicator for *Candida albicans* biofilm formation and drug resistance. *Frontiers in Microbiology*, 12, 655242.
- Denning, D. W., Kneale, M., Sobel, J. D., & Rautemaa-Richardson, R. (2018). Global burden of recurrent vulvovaginal candidiasis: a systematic review. *The Lancet infectious diseases*, 18(11), e339-e347.
- Deorukhkar, S. and Saini, S. (2013). Evaluation of phospholipase activity in biofilm forming *Candida* species isolated from intensive care unit patients. *Microbiology Research Journal International*, 440-447.
- Döğen, A., Gümrül, R., & Ilkit, M. (2015). Haemolytic and co-haemolytic (CAMP-like) activity in dermatophytes. *Mycoses*, 58(1), 40-47.
- Doron, I., Leonardi, I., Li, X. V., Fiers, W. D., Semon, A., Bialt-DeCelie, M., ... & Iliev, I. D. (2021). Human gut mycobiota tune immunity via CARD9-dependent induction of anti-fungal IgG antibodies. *Cell*, 184(4), 1017-1031.
- Douglas, L. J. (2002). Medical importance of biofilms in *Candida* infections. *Revista iberoamericana de micologia*, 19(3), 139-143.
- Downes, F. and Ito, K. (2001). *Compendium of Methods for the Microbiological Examination of Foods*. Washington DC, USA: American Public Health Association.
- Dudek, B., Warskulat, A. C., & Schneider, B. (2016). The occurrence of flavonoids and related compounds in flower sections of *Papaver nudicaule*. *Plants*, 5(2), 28.
- Edward, F. G. (1999). Fact Sheet FPS-152. *Series of the Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida*.
- Egamberdieva, D., Mamedov, N., Ovidi, E., Tiezzi, A., & Craker, L. (2017). Phytochemical and pharmacological properties of medicinal plants from Uzbekistan: A review. *Journal of Medicinally Active Plants*, 5(2), 59-75.
- Ernst, M., Saslis-Lagoudakis, C. H., Grace, O. M., Nilsson, N., Simonsen, H. T., Horn, J. W., & Rønsted, N. (2016). Evolutionary prediction of medicinal properties in the genus *Euphorbia* L. *Scientific Reports*, 6(1), 1-10.

References

- Fang, R. L., Chen, L. X., Shu, W. S., Yao, S. Z., Wang, S. W., & Chen, Y. Q. (2016). Barcoded sequencing reveals diverse intrauterine microbiomes in patients suffering with endometrial polyps. *American journal of translational research*, 8(3), 1581.
- Farmakiotis, D., Tarrand, J. J., & Kontoyiannis, D. P. (2014). Drug-resistant *Candida glabrata* infection in cancer patients. *Emerging infectious diseases*, 20(11), 1833.
- Felix, T. C., de Brito Röder, D. V. D., & dos Santos Pedroso, R. (2019). Alternative and complementary therapies for vulvovaginal candidiasis. *Folia microbiologica*, 64(2), 133-141.
- Firenzuoli, F., & Gori, L. (2007). Herbal medicine today: Clinical and Research Issues. *eCAM*, 4.
- Fotedar, R., & Al-Hedaithy, S. S. A. (2005). Comparison of phospholipase and proteinase activity in *Candida albicans* and *C. dubliniensis*. *Mycoses*, 48(1), 62-67.
- Furlaneto, M. C., Góes, H. P., Perini, H. F., Dos Santos, R. C., & Furlaneto-Maia, L. (2018). How much do we know about hemolytic capability of pathogenic *Candida* species?. *Folia microbiologica*, 63(4), 405-412.
- Garcia-Rubio, R., de Oliveira, H. C., Rivera, J., & Trevijano-Contador, N. (2020). The fungal cell wall: *Candida*, *Cryptococcus*, and *Aspergillus* species. *Frontiers in microbiology*, 10, 2993.
- George, K. W., Alonso-Gutierrez, J., Keasling, J. D., & Lee, T. S. (2015). Isoprenoid drugs, biofuels, and chemicals—artemisinin, farnesene, and beyond. *Biotechnology of isoprenoids*, 355-389.
- Ghazal, A. R. A., Idris, G., Hajeer, M. Y., Alawer, K., & Cannon, R. D. (2019). Efficacy of removing *Candida albicans* from orthodontic acrylic bases: an in vitro study. *BMC oral health*, 19(1), 1-7.
- Gonzalez-Lara, M. F., & Ostrosky-Zeichner, L. (2020, February). Invasive candidiasis. In *Seminars in Respiratory and Critical Care Medicine* (Vol. 41, No. 01, pp. 003-012). Thieme Medical Publishers.
- Grzegocka, K., Krzyściak, P., Hille-Padalis, A., Loster, J. E., Talaga-Ćwiertnia, K., & Loster, B. W. (2020). *Candida* prevalence and oral hygiene due to orthodontic therapy with conventional brackets. *BMC Oral Health*, 20(1), 1-9.

References

- Guerriero, G., Berni, R., Muñoz-Sanchez, J. A., Apone, F., Abdel-Salam, E. M., Qahtan, A. A., ... & Faisal, M. (2018). Production of plant secondary metabolites: Examples, tips and suggestions for biotechnologists. *Genes*, 9(6), 309.
- Guinea, J. (2014). Global trends in the distribution of *Candida* species causing candidemia. *Clinical Microbiology and Infection*, 20, 5-10.
- Guo, X., Zheng, M., Pan, R., Zang, B., Gao, J., Ma, H., & Jin, M. (2019). Hydroxysafflor yellow A (HSYA) targets the platelet-activating factor (PAF) receptor and inhibits human bronchial smooth muscle activation induced by PAF. *Food & Function*, 10(8), 4661-4673.
- Gutiérrez J, Morales P, González MA, Quindós G. *Candida dubliniensis*, a new fungal pathogen. *J Basic Microbiol.* (2002) 42:207–27. doi: 10.1002/1521-4028(200206)42:3<207::AID-JOBM207>3.0.CO;2-C
- Haggerty, C. L., Hillier, S. L., Bass, D. C., Ness, R. B., & PID Evaluation and Clinical Health (PEACH) Study Investigators. (2004). Bacterial vaginosis and anaerobic bacteria are associated with endometritis. *Clinical Infectious Diseases*, 39(7), 990-995.
- Hall, R. A. (2015). Dressed to impress: impact of environmental adaptation on the *Candida albicans* cell wall. *Molecular microbiology*, 97(1), 7-17.
- Hamilton, A. C. (2004). Medicinal plants, conservation and livelihoods. *Biodiversity & Conservation*, 13(8), 1477-1517.
- Hamilton, A. C. (2004). Medicinal plants, conservation and livelihoods. *Biodiversity & Conservation*, 13(8), 1477-1517.
- Haque, E., Irfan, S., Kamil, M., Sheikh, S., Hasan, A., Ahmad, A., ... & Mir, S. S. (2016). Terpenoids with antifungal activity trigger mitochondrial dysfunction in *Saccharomyces cerevisiae*. *Microbiology*, 85(4), 436-443.
- Harborne, J.B, Mabray TY, Marby H. *Physiology and function of flavonoids*. Academic Press, New York, 1975; pp 970.
- Hart, G. (1993). Factors associated with trichomoniasis, candidiasis and bacterial vaginosis. *International journal of STD & AIDS*, 4(1), 21-25.
- Hasan Radhi, S., Kamal, S. A., Mohammed Sahi, N., & Hussein, H. J. (2022). Assessment of Antibacterial Efficacy of *Callistemon viminalis* (Sol.

References

- ex Gaertn.) G. Don against Some Isolates Obtained from Urinary Tract Infections. Archives of Razi Institute, 77(2), 891-897.
- Hawksworth, D. L. (2001). The magnitude of fungal diversity: the 1• 5 million species estimate revisited. Mycological research, 105(12), 1422-1432.
 - Healey, K. R., Zhao, Y., Perez, W. B., Lockhart, S. R., Sobel, J. D., Farmakiotis, D., ... & Perlin, D. S. (2016). Prevalent mutator genotype identified in fungal pathogen *Candida glabrata* promotes multi-drug resistance. Nature communications, 7(1), 1-10.
 - Hectors, K., Van Oevelen, S., Geuns, J., Guisez, Y., Jansen, M. A., & Prinsen, E. (2014). Dynamic changes in plant secondary metabolites during UV acclimation in *Arabidopsis thaliana*. Physiologia Plantarum, 152(2), 219-230.
 - Heidari, M. F., Arab, S. S., Noroozi-Aghideh, A., Tebyanian, H., & Latifi, A. M. (2019). Evaluation of the substitutions in 212, 342 and 215 amino acid positions in binding site of organophosphorus acid anhydrolase using the molecular docking and laboratory analysis. Bratislava Medical Journal- Bratislavske Lekarske Listy, 120(2), 139-143.
 - Höfs, S., Mogavero, S., & Hube, B. (2016). Interaction of *Candida albicans* with host cells: virulence factors, host defense, escape strategies, and the microbiota. Journal of microbiology, 54(3), 149-169.
 - Horn, D. L., Neofytos, D., Anaissie, E. J., Fishman, J. A., Steinbach, W. J., Olyaei, A. J., ... & Webster, K. M. (2009). Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clinical infectious diseases, 48(12), 1695-1703.
 - Horn, D. L., Neofytos, D., Anaissie, E. J., Fishman, J. A., Steinbach, W. J., Olyaei, A. J., ... & Webster, K. M. (2009). Epidemiology and outcomes of candidemia in 2019 patients: data from the prospective antifungal therapy alliance registry. Clinical infectious diseases, 48(12), 1695-1703 .
 - Houchi, S., & Messasma, Z. (2022). Exploring the inhibitory potential of *Saussurea costus* and *Saussurea involucreta* phytoconstituents against the Spike glycoprotein receptor binding domain of SARS-CoV-2 Delta (B. 1.617. 2) variant and the main protease (Mpro) as therapeutic candidates,

References

- using Molecular docking, DFT, and ADME/Tox studies. *Journal of Molecular Structure*, 1263, 133032.
- Hsueh, P. R., Graybill, J. R., Playford, E. G., Watcharananan, S. P., Oh, M. D., Ja'alam, K., ... & Padiglione, A. A. (2009). Consensus statement on the management of invasive candidiasis in Intensive Care Units in the Asia-Pacific Region. *International journal of antimicrobial agents*, 34(3), 205-209.
 - Huang, H. (2011). Plant diversity and conservation in China: planning a strategic bioresource for a sustainable future. *Botanical Journal of the Linnean Society*, 166(3), 282-300.
 - Hudzicki, J. (2009). Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology*, 15, 55-63.
 - Hussain, A. Y., Hussein, H. J., & Al-Rubaye, A. F. (2021). Antifungal Activity of the Secondary Metabolites Extracted from *Carthamus tinctorius* L. against *Aspergillus* Species Isolated from Stored Medicinal Plants Seeds in the Iraqi Markets. *Clinical Schizophrenia & Related Psychoses*.
 - Hussain, A. Y., Hussein, H. J., & Al-Rubaye, A. F. (2021). Antifungal Efficacy of the crude Flavonoid, Terpenoid, and Alkaloid Extracted from *Myrtus communis* L. against *Aspergillus* species isolated from Stored Medicinal plants seeds in the Iraqi Markets. *Journal of Biotechnology Research Center*, 15(2), 73-80.
 - Hussain, A. Y., Hussein, H. J., & Al-Rubaye, A. F. (2021). Antifungal Efficacy of the crude Flavonoid, Terpenoid, and Alkaloid Extracted from *Myrtus communis* L. against *Aspergillus* species isolated from Stored Medicinal plants seeds in the Iraqi Markets. *Journal of Biotechnology Research Center*, 15(2), 73-80.
 - Hussein, H. J., & Al-Marzoqi, A. H. (2020). The Antibacterial efficacy of the secondary metabolites extracted from (*Melia azedarach* L.) leaves against pathogenic microorganisms isolated from burns and gingivitis infections. *EurAsian Journal of Biosciences*, 14(1), 561-565.
 - Hussein, H. J., Kamal, S. A., & Sahi, N. M. (2020). Antibacterial efficacy of the seed extract of *salvia hispanica* L. against pathogenic bacteria isolated from diarrhea cases. *Biochem Cell Arch*, 20(2), 3491-4.

References

- Ilkit, M., & Guzel, A. B. (2011). The epidemiology, pathogenesis, and diagnosis of vulvovaginal candidosis: a mycological perspective. *Critical reviews in microbiology*, 37(3), 250-261.
- Jack, D. B. (1997). One hundred years of aspirin. *The Lancet*, 350(9075), 437-439.
- Jensen, R. H., Astvad, K. M. T., Silva, L. V., Sanglard, D., Jørgensen, R., Nielsen, K. F., ... & Arendrup, M. C. (2015). Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance in vivo in *Candida albicans* orchestrated by multiple genetic alterations. *Journal of Antimicrobial Chemotherapy*, 70(9), 2551-2555.
- Jensen, R. H., Justesen, U. S., Rewes, A., Perlin, D. S., & Arendrup, M. C. (2014). Echinocandin failure case due to a previously unreported FKS1 mutation in *Candida krusei*. *Antimicrobial agents and chemotherapy*, 58(6), 3550-3552.
- Jiang, N., Doseff, A. I., & Grotewold, E. (2016). Flavones: from biosynthesis to health benefits. *Plants*, 5(2), 27.
- Johnson, T. O., Ermolieff, J., & Jirousek, M. R. (2002). Protein tyrosine phosphatase 1B inhibitors for diabetes. *Nature Reviews Drug Discovery*, 1(9), 696-709.
- Jordá, T., & Puig, S. (2020). Regulation of ergosterol biosynthesis in *Saccharomyces cerevisiae*. *Genes*, 11(7), 795.
- Kamal, A. S., Hussein, H. J., & Tolaifeh, Z. A. (2019). Antibacterial potential of *Hibiscus sabdarifa* L. against some Enterobacteriaceae: in vitro. *Biochemical and cellular archives*, 19(2), 4291-4294.
- Kamalpreet, L. K., Singh, A., Kaur, J., & Kaur, N. (2019). A brief review of remedial uses of *Saussurea lappa*. *Journal of Pharmacognosy and Phytochemistry*, 8(3), 4423-4430.
- Kamil, S. S., Hussein, H. J., & Al-Marzoqi, A. H. (2020). Evolution of Antibacterial efficacy of *Dianthus caryophyllus* L. extracts against some hospitals pathogenic bacteria. *International Journal of Pharmaceutical Research*, 12(3), 1274-1279.
- Kari, E., Miettinen, P., Yli-Pirilä, P., Virtanen, A., & Faiola, C. L. (2018). PTR-ToF-MS product ion distributions and humidity-dependence of biogenic

References

- volatile organic compounds. *International Journal of Mass Spectrometry*, 430, 87-97.
- Kaul MK. Medicinal plants of Kashmir and Ladakh: temperate and cold arid Himalaya. Indus publishing; 1997.
 - Khan, Z., Ahmad, S., Joseph, L., & Chandy, R. (2012). *Candida dubliniensis*: an appraisal of its clinical significance as a bloodstream pathogen. *PLoS One*, 7(3), e32952.
 - Khémiri, I., Essghaier, B., Sadfi-Zouaoui, N., & Bitri, L. (2020). Antioxidant and antimicrobial potentials of seed oil from *Carthamus tinctorius* L. in the management of skin injuries. *Oxidative Medicine and Cellular Longevity*, 2020.
 - Kočendová, J., Vaňková, E., Volejníková, A., Nešuta, O., Buděšínský, M., Socha, O., ... & Čerovský, V. (2019). Antifungal activity of analogues of antimicrobial peptides isolated from bee venoms against vulvovaginal *Candida* spp. *FEMS yeast research*, 19(3), foz013.
 - Komarov, V. L., & Shishkin, B. K. (1970). *Flora of the USSR Centrospermae.—Vol. VI*, Translated from Russian by N. Landau, Jerusalem.
 - Konje, J. C., Otolorin, E. O., Ogunniyi, J. O., Obisesan, K. A., & Ladipo, O. A. (1991). The prevalence of *Gardnerella vaginalis*, *Trichomonas vaginalis* and *Candida albicans* in the cytology clinic at Ibadan, Nigeria. *African journal of medicine and medical sciences*, 20(1), 29-34.
 - Kornitzer, D. (2019). Regulation of *Candida albicans* hyphal morphogenesis by endogenous signals. *Journal of Fungi*, 5(1), 21.
 - Kornitzer, D. (2019). Regulation of *Candida albicans* hyphal morphogenesis by endogenous signals. *Journal of Fungi*, 5(1), 21.
 - Kruawan, K., & Kangsadalampai, K. (2006). Antioxidant activity, phenolic compound contents and antimutagenic activity of some water extract of herbs. *Thai J Pharm Sci*, 30(1), 28-35.
 - Kubeczka, K. H. (2020). History and sources of essential oil research. In *Handbook of essential oils* (pp. 3-39). CRC Press.
 - Kumar, S. P., & Kumari, B. R. (2011). Factors affecting on somatic embryogenesis of safflower (*Carthamus tinctorius* L) at morphological and biochemical levels. *World J Agric Sci*, 7(2), 197-205.

References

- Lagree, K., Desai, J. V., Finkel, J. S., & Lanni, F. (2018). Microscopy of fungal biofilms. *Current opinion in microbiology*, 43, 100-107.
- Lamoth, F., Lockhart, S. R., Berkow, E. L., & Calandra, T. (2018). Changes in the epidemiological landscape of invasive candidiasis. *Journal of Antimicrobial Chemotherapy*, 73(suppl_1), i4-i13.
- Lange, B. M., & Ahkami, A. (2013). Metabolic engineering of plant monoterpenes, sesquiterpenes and diterpenes—current status and future opportunities. *Plant biotechnology journal*, 11(2), 169-196.
- Lehmann, P. F. (2010). Fungal structure and morphology. *Topley & Wilson's Microbiology and Microbial Infections*.
- Li, Y., Kong, D., Fu, Y., Sussman, M. R., & Wu, H. (2020). The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*, 148, 80-89.
- Li, Y., Xu, C., Zhang, Q., Liu, J. Y., & Tan, R. X. (2005). In vitro anti-*Helicobacter pylori* action of 30 Chinese herbal medicines used to treat ulcer diseases. *Journal of ethnopharmacology*, 98(3), 329-333.
- Lim TK. Edible medicinal and non-medicinal plants. Dordrecht, the Netherlands: Springer; 2012.
- Limia, O. F., & Lantero, M. I. (2004). Prevalence of *Candida albicans* and *Trichomonas vaginalis* in pregnant women in Havana City by an immunologic latex agglutination test. *Medscape General Medicine*, 6(4).
- Lindberg Madsen, H., & Bertelsen, G. (1995). Spices as antioxidants. *Trends in Food Science & Technology*, 6(8), 271-74.
- Lionakis, M. S., & Hohl, T. M. (2020). Call to action: how to tackle emerging nosocomial fungal infections. *Cell host & microbe*, 27(6), 859-862.
- Lohse, M. B., Gulati, M., Johnson, A. D., & Nobile, C. J. (2018). Development and regulation of single-and multi-species *Candida albicans* biofilms. *Nature Reviews Microbiology*, 16(1), 19-31.
- Lohse, M. B., Gulati, M., Johnson, A. D., & Nobile, C. J. (2018). Development and regulation of single-and multi-species *Candida albicans* biofilms. *Nature Reviews Microbiology*, 16(1), 19-31.
- Ludwiczuk, A., Skalicka-Woźniak, K., & Georgiev, M. I. (2017). Terpenoids. In *Pharmacognosy* (pp. 233-266). Academic Press.

References

- Madhuri, G., & Barad, A. V. (2018). Effect of NPK nutrients through foliar spray on vegetative growth parameters of carnation (*Dianthus caryophyllus* L.) varieties under protected condition. *The Pharma Innovation*, 7(5, Part H), 559.
- Mahood, H. E., Alwash, B. M., & Ibrahim, K. M. (2018). Improvement of alkaloids yield using phenylalanine as a precursor supplemented to MORINA oleifera L. callus cultures. *Biochemical and Cellular Archives*, 18(Suppl. 1), 913-919.
- Makanjuola, O., Bongomin, F., & Fayemiwo, S. A. (2018). An update on the roles of non-albicans *Candida* species in vulvovaginitis. *Journal of Fungi*, 4(4), 121.
- Martin Jr, H. L., Richardson, B. A., Nyange, P. M., Lavreys, L., Hillier, S. L., Chohan, B., ... & Kreiss, J. (1999). Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. *Journal of Infectious Diseases*, 180(6), 1863-1868.
- Martin, V. (2003). JJ, Pitera, DJ, Withers, ST, Newman, JD & Keasling, JD Engineering a mevalonate pathway in *Escherichia coli* for production of terpenoids. *Nature Biotech*, 21, 796-802.
- McCall, A. D., Pathirana, R. U., Prabhakar, A., Cullen, P. J., & Edgerton, M. (2019). *Candida albicans* biofilm development is governed by cooperative attachment and adhesion maintenance proteins. *NPJ biofilms and microbiomes*, 5(1), 1-12.
- McCarty, T. P., & Pappas, P. G. (2016). Invasive candidiasis. *Infectious Disease Clinics*, 30(1), 103-124.
- McCarty, T. P., White, C. M., & Pappas, P. G. (2021). Candidemia and invasive candidiasis. *Infectious Disease Clinics*, 35(2), 389-413.
- McCarty, T. P., White, C. M., & Pappas, P. G. (2021). Candidemia and invasive candidiasis. *Infectious Disease Clinics*, 35(2), 389-413.
- Molero, G., Diez-Orejas, R., Navarro-Garcia, F., Monteoliva, L., Pla, J., Gil, C., ... & Nombela, C. (1998). *Candida albicans*: genetics, dimorphism and pathogenicity. *International Microbiology*, 1(2), 95-106.
- Molero, G., Diez-Orejas, R., Navarro-Garcia, F., Monteoliva, L., Pla, J., Gil, C., ... & Nombela, C. (1998). *Candida albicans*: genetics, dimorphism and pathogenicity. *International Microbiology*, 1(2), 95-106.

References

- Monif, G. R. (2001). Diagnosis of infectious vulvovaginal disease. *Infections in Medicine*, 18(12), 532-533.
- Moran, G. P. *Candida albicans* versus *Candida dubliniensis*: Why Is *C. albicans* More Pathogenic. *Int J Microbiol*, (2012), 205921.
- Morio, F., Jensen, R. H., Le Pape, P., & Arendrup, M. C. (2017). Molecular basis of antifungal drug resistance in yeasts. *International journal of antimicrobial agents*, 50(5), 599-606.
- Mtibaa, L., Fakhfakh, N., Kallel, A., Belhadj, S., Salah, N. B., Bada, N., & Kallel, K. (2017). Vulvovaginal candidiasis: Etiology, symptomatology and risk factors. *Journal de mycologie medicale*, 27(2), 153-158.
- Mukhia, K. R., & Urhekar, A. D. (2016). Biofilm production by various *Candida* species isolated from various clinical specimens. *Int J Sci Res*, 5, 2388-92.
- Nadeem, S.G.; Hakim, S.T. and Kazmi, S.U.(2010).Use of CHROMagar *Candida* for the presumptive identification of *Candida* species directly from clinical specimens in resource-limited settings.*Libyan Journal of Medicine* 5.
- Nadkarni KM. *Indian materia medica*, popular Book Depot. Bombay. 1954;7:946-8.
- Nadkarni, K. M. (2004). *Medicinal plants of India*. Reprint Publications.
- Navarro, M., Moreira, I., Arnaez, E., Quesada, S., Azofeifa, G., Vargas, F., ... & Chen, P. (2017). Flavonoids and ellagitannins characterization, antioxidant and cytotoxic activities of *Phyllanthus acuminatus* Vahl. *Plants*, 6(4), 62.
- Neves, E. O., Sales, P. M. D., & Silveira, D. (2022). Post-marketing sampling and testing programs for licensed medicinal products: a narrative review. *Brazilian Journal of Pharmaceutical Sciences*, 58.
- Nucci, M., & Colombo, A. L. (2007). Candidemia due to *Candida tropicalis*: clinical, epidemiologic, and microbiologic characteristics of 188 episodes occurring in tertiary care hospitals. *Diagnostic microbiology and infectious disease*, 58(1), 77-82.
- Okusa Ndjolo, P., Stevigny, C., Duez, P., Varela, A., & Ibañez, J. (2009). Medicinal plants: A tool to overcome antibiotic resistance?. *Medicinal plants: classification, biosynthesis and pharmacology*, 315-336.

References

- Orlandi, V. T., Martegani, E., & Bolognese, F. (2018). Catalase A is involved in the response to photooxidative stress in *Pseudomonas aeruginosa*. *Photodiagnosis and Photodynamic Therapy*, 22, 233-240.
- Ost, K. S., O'Meara, T. R., Stephens, W. Z., Chiaro, T., Zhou, H., Penman, J., ... & Round, J. L. (2021). Adaptive immunity induces mutualism between commensal eukaryotes. *Nature*, 596(7870), 114-118.
- Paddon, C. J., & Keasling, J. D. (2014). Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. *Nature reviews microbiology*, 12(5), 355-367.
- Pandey MM, Rastogi S, Rawat AK. *Saussurea costus*: botanical, chemical and pharmacological review of an ayurvedic medicinal plant. *Journal of ethnopharmacology*. 2007 Apr 4;110(3):379-90.
- Pappas, P. G., & Lionakis, M. S. (2018). Arendrup MCet al. Invasive candidiasis. *Nat Rev Dis Primers*, 4, 18026.
- Pappas, P. G., Lionakis, M. S., Arendrup, M. C., Ostrosky-Zeichner, L., & Kullberg, B. J. (2018). Invasive candidiasis. *Nature Reviews Disease Primers*, 4(1), 1-20.
- Parekh, J., & Chanda, S. (2008). In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. *African journal of Biotechnology*, 7(23).
- Parekh, J., & Chanda, S. (2008). In vitro antifungal activity of methanol extracts of some Indian medicinal plants against pathogenic yeast and moulds. *African journal of Biotechnology*, 7(23).
- Peixoto, F., Camargos, A., Duarte, G., Linhares, I., Bahamondes, L., & Petracco, A. (2008). Efficacy and tolerance of metronidazole and miconazole nitrate in treatment of vaginitis. *International Journal of Gynecology & Obstetrics*, 102(3), 287-292.
- Pépin, J., Deslandes, S., Giroux, G., Sobéla, F., Khonde, N., Diakité, S., ... & Frost, E. (2011). The complex vaginal flora of West African women with bacterial vaginosis. *PloS one*, 6(9), e25082.
- Perveen, S., & Al-Taweel, A. (Eds.). (2018). *Terpenes and terpenoids*. BoD—Books on Demand.

References

- Pfaller, M. A., & Diekema, D. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clinical microbiology reviews*, 20(1), 133-163.
- Pfaller, M. A., & Diekema, D. (2007). Epidemiology of invasive candidiasis: a persistent public health problem. *Clinical microbiology reviews*, 20(1), 133-163.
- Pfaller, M. A., Diekema, D. J., Gibbs, D. L., Newell, V. A., Ellis, D., Tullio, V., ... & Ling, T. A. (2010). Results from the ARTEMIS DISK Global Antifungal Surveillance Study, 1997 to 2007: a 10.5-year analysis of susceptibilities of *Candida* species to fluconazole and voriconazole as determined by CLSI standardized disk diffusion. *Journal of clinical microbiology*, 48(4), 1366-1377.
- Pfaller, M. A., Moet, G. J., Messer, S. A., Jones, R. N., & Castanheira, M. (2011). Geographic variations in species distribution and echinocandin and azole antifungal resistance rates among *Candida* bloodstream infection isolates: report from the SENTRY Antimicrobial Surveillance Program (2008 to 2009). *Journal of clinical microbiology*, 49(1), 396-399 .
- Pfaller, M. A., Moet, G. J., Messer, S. A., Jones, R. N., & Castanheira, M. (2011). Geographic variations in species distribution and echinocandin and azole antifungal resistance rates among *Candida* bloodstream infection isolates: report from the SENTRY Antimicrobial Surveillance Program (2008 to 2009). *Journal of clinical microbiology*, 49(1), 396-399 .
- Pfaller, M. A., Moet, G. J., Messer, S. A., Jones, R. N., & Castanheira, M. (2011). Geographic variations in species distribution and echinocandin and azole antifungal resistance rates among *Candida* bloodstream infection isolates: report from the SENTRY Antimicrobial Surveillance Program (2008 to 2009). *Journal of clinical microbiology*, 49(1), 396-399 .
- Phillipson, J. D. (2001). Phytochemistry and medicinal plants. *Phytochemistry*, 56(3), 237-243.
- Pinto, L. M., Neto, F. D. A. B., de Medeiros, M. A. P., Alves, D. L. Z., & Chaves, G. M. (2019). *Candida* species isolated from pigeon (*Columba livia*) droppings may express virulence factors and resistance to azoles. *Veterinary microbiology*, 235, 43-52.

References

- Posser, J., Girardi, J. P., Pedroso, D., & Sandri, Y. P. (2015). Estudo das infecções cervicovaginais diagnosticadas pela citologia. *Revista Saúde Integrada*, 8, 15-16.
- Pramanick, R., Mayadeo, N., Warke, H., Begum, S., Aich, P., & Aranha, C. (2019). Vaginal microbiota of asymptomatic bacterial vaginosis and vulvovaginal candidiasis: Are they different from normal microbiota?. *Microbial pathogenesis*, 134, 103599.
- Primon-Barros, M., Rigo, G. V., Frasson, A. P., Santos, O. D., Smiderle, L., Almeida, S., ... & Tasca, T. (2015). Modulatory effect of iron chelators on adenosine deaminase activity and gene expression in *Trichomonas vaginalis*. *Memórias do Instituto Oswaldo Cruz*, 110, 877-883.
- Pristov, K. E., & Ghannoum, M. A. (2019). Resistance of *Candida* to azoles and echinocandins worldwide. *Clinical Microbiology and Infection*, 25(7), 792-798.
- Pristov, K. E., & Ghannoum, M. A. (2019). Resistance of *Candida* to azoles and echinocandins worldwide. *Clinical Microbiology and Infection*, 25(7), 792-798.
- Priya, A., & Pandian, S. K. (2020). Piperine impedes biofilm formation and hyphal morphogenesis of *Candida albicans*. *Frontiers in microbiology*, 11, 756.
- Punjanon, T., Arpornsuwan, T., & Klinkusoom, N. (2004). The pharmacological properties of safflower (*Carthamus tinctorius* L). *Bulletin of Health Science and Technology*, 7, 51-63.
- Qiu, J. (2007). Traditional medicine: a culture in the balance. *Nature*, 448(7150), 126-129.
- Rafieian-Kopaei, M. (2012). Medicinal plants and the human needs. *Journal of HerbMed Pharmacology*, 1.
- Rao, C., Coyte, K. Z., Bainter, W., Geha, R. S., Martin, C. R., & Rakoff-Nahoum, S. (2021). Multi-kingdom ecological drivers of microbiota assembly in preterm infants. *Nature*, 591(7851), 633-638.
- Rao, K. S., Babu, G. V., & Ramnareddy, Y. V. (2007). Acylated flavone glycosides from the roots of *Saussurea lappa* and their antifungal activity. *Molecules*, 12(3), 328-344.

References

- Rao, K. S., Babu, G. V., & Ramnareddy, Y. V. (2007). Acylated flavone glycosides from the roots of *Saussurea lappa* and their antifungal activity. *Molecules*, 12(3), 328-344.
- Rauter, A. P., Ennis, M., Hellwich, K. H., Herold, B. J., Horton, D., Moss, G. P., & Schomburg, I. (2018). Nomenclature of flavonoids (IUPAC recommendations 2017). *Pure and Applied Chemistry*, 90(9), 1429-1486.
- Reyna-Beltrán, E., Isaac Bazán Méndez, C., Iranzo, M., Mormeneo, S., & Pedro Luna-Arias, J. (2019). The cell wall of *Candida albicans*: A proteomics view. *Candida albicans*, 12, 71-92.
- Robinson, L. S., Perry, J., Lek, S., Wollam, A., Sodergren, E., Weinstock, G., ... & Lewis, A. L. (2016). Genome sequences of 15 *Gardnerella vaginalis* strains isolated from the vaginas of women with and without bacterial vaginosis. *Genome announcements*, 4(5), e00879-16.
- Rodrigues, C. F., Rodrigues, M. E., Silva, S., & Henriques, M. (2017). *Candida glabrata* biofilms: how far have we come?. *Journal of fungi*, 3(1), 11.
- Rodrigues, M. T., Gonçalves, A. C., Alvim, M. C. T., Castellano Filho, D. S., Zimmermann, J. B., Silva, V. L. D., & Diniz, C. G. (2013). Associação entre cultura de secreção vaginal, características sociodemográficas e manifestações clínicas de pacientes com diagnóstico de candidíase vulvovaginal. *Revista Brasileira de Ginecologia e Obstetrícia*, 35, 554-561.
- Rodríguez-Cerdeira, C., Gregorio, M. C., Molares-Vila, A., López-Barcenas, A., Fabbrocini, G., Bardhi, B., ... & Hernandez-Castro, R. (2019). Biofilms and vulvovaginal candidiasis. *Colloids and Surfaces B: Biointerfaces*, 174, 110-125.
- Rosa, M. I., Silva, B. R., Pires, P. S., Silva, F. R., Silva, N. C., Souza, S. L., ... & Medeiros, L. R. (2013). Weekly fluconazole therapy for recurrent vulvovaginal candidiasis: a systematic review and meta-analysis. *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 167(2), 132-136.
- Sachin, C.; Ruchi, K. and Santosh, S. (2012). In vitro evaluation of proteinase, phospholipase and haemolysin activities of *Candida* species isolated from clinical specimens. *International journal of Medicine and Biomedical research* 1: 153-157.

References

- Salem, N., Msaada, K., Elkahoui, S., Mangano, G., Azaeiz, S., Ben Slimen, I., ... & Marzouk, B. (2014). Evaluation of antibacterial, antifungal, and antioxidant activities of safflower natural dyes during flowering. *BioMed research international*, 2014.
- Salminen, K. A., Meyer, A., Jerabkova, L., Korhonen, L. E., Rahnasto, M., Juvonen, R. O., ... & Raunio, H. (2011). Inhibition of human drug metabolizing cytochrome P450 enzymes by plant isoquinoline alkaloids. *Phytomedicine*, 18(6), 533-538.
- Saxena, M., Saxena, J., Nema, R., Singh, D., & Gupta, A. (2013). Phytochemistry of medicinal plants. *Journal of pharmacognosy and phytochemistry*, 1(6).
- Schippmann, U. W. E., Leaman, D., & Cunningham, A. B. (2006). A comparison of cultivation and wild collection of medicinal and aromatic plants under sustainability aspects. *Frontis*, 75-95.
- Segal, E., & Frenkel, M. (2018). Experimental in vivo models of candidiasis. *Journal of fungi*, 4(1), 21.
- Shao, T. Y., Ang, W. G., Jiang, T. T., Huang, F. S., Andersen, H., Kinder, J. M., ... & Way, S. S. (2019). Commensal *Candida albicans* positively calibrates systemic Th17 immunological responses. *Cell host & microbe*, 25(3), 404-417.
- Shapiro, H. (2006). *Medicine across cultures: history and practice of medicine in non-western cultures (Vol. 3)*. Springer Science & Business Media.
- Sharara, D. T., Al-Marzoqi, A. H., & Hussein, H. J. (2021). In Vitro Antibacterial efficacy of the Secondary Metabolites Extracted from *Myrtus communis* L. against some pathogenic bacteria isolated from Hemodialysis Fluid. *Annals of the Romanian Society for Cell Biology*, 25(6), 9267-9274.
- Sharkey, T. D., Wiberley, A. E., & Donohue, A. R. (2008). Isoprene emission from plants: why and how. *Annals of botany*, 101(1), 5-18.
- Shipitsyna, E., Roos, A., Datcu, R., Hallén, A., Fredlund, H., Jensen, J. S., ... & Unemo, M. (2013). Composition of the vaginal microbiota in women of reproductive age—sensitive and specific molecular diagnosis of bacterial vaginosis is possible?. *PloS one*, 8(4), e60670.

References

- Shirwaikar, A., Khan, S., Kamariya, Y. H., Patel, B. D., & Gajera, F. P. (2010). Medicinal plants for the management of post menopausal osteoporosis: A review. *The Open Bone Journal*, 2(1).
- Shokralla, S., Spall, J. L., Gibson, J. F., & Hajibabaei, M. (2012). Next-generation sequencing technologies for environmental DNA research. *Molecular ecology*, 21(8), 1794-1805.
- Simmons, C. W., Reddy, A. P., Simmons, B. A., Singer, S. W., & VanderGheynst, J. S. (2014). Effect of inoculum source on the enrichment of microbial communities on two lignocellulosic bioenergy crops under thermophilic and high-solids conditions. *Journal of applied microbiology*, 117(4), 1025-1034.
- Singh, A., & Pandey, A. K. (2021). Naturally biosynthesized secondary plant products and its importance: An overview.
- Singh, D. K., Tóth, R., & Gácsér, A. (2020). Mechanisms of pathogenic *Candida* species to evade the host complement attack. *Frontiers in cellular and infection microbiology*, 10, 94.
- Singh, J. S. (2002). The biodiversity crisis: a multifaceted review. *Current Science*, 638-647.
- Singh, J. S. (2002). The biodiversity crisis: a multifaceted review. *Current Science*, 638-647.
- Sivakumar, V.; Shankar, P.; Nalina, K.; Menon, T.(2009). Use of CHROMagar in the differentiation of common species of *Candida*. *Mycopathologia* 167: 47-49.
- Siwko, M. E., Marrink, S. J., de Vries, A. H., Kozubek, A., Uiterkamp, A. J. S., & Mark, A. E. (2007). Does isoprene protect plant membranes from thermal shock? A molecular dynamics study. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1768(2), 198-206.
- Smith, P. B., Morgan, J., Benjamin, D. K., Fridkin, S. K., Sanza, L. T., Harrison, L. H., ... & Benjamin Jr, D. K. (2007). Excess costs of hospital care associated with neonatal candidemia. *The Pediatric infectious disease journal*, 26(3), 197-200.
- Sobel JD. Vulvovaginal candidosis. *Lancet*. 2007;369:1961–1971. [PubMed] [Google Scholar]

References

- Sobel, J. D. (2007). Vulvovaginal candidosis. *The Lancet*, 369(9577), 1961-1971.
- Solecki, R. S. (1975). Shanidar IV, a Neanderthal flower burial in northern Iraq. *Science*, 190(4217), 880-881.
- Soliman, M. F., Shetaia, Y. M., Tayel, A. A., Munshi, A. M., Alatawi, F. A., Alsieni, M. A., & Al-Saman, M. A. (2022). Exploring the Antifungal Activity and Action of *Saussurea costus* Root Extracts against *Candida albicans* and Non-*albicans* Species. *Antibiotics*, 11(3), 327.
- Soliman, M. F., Shetaia, Y. M., Tayel, A. A., Munshi, A. M., Alatawi, F. A., Alsieni, M. A., & Al-Saman, M. A. (2022). Exploring the Antifungal Activity and Action of *Saussurea costus* Root Extracts against *Candida albicans* and Non-*albicans* Species. *Antibiotics*, 11(3), 327.
- Souza, G. N. D., Vieira, T. C. S. B., Campos, A. A. S., Leite, A. P. L., & Souza, E. D. (2012). Tratamento das vulvovaginites na gravidez. *Femina* .
- Souza, G. N. D., Vieira, T. C. S. B., Campos, A. A. S., Leite, A. P. L., & Souza, E. D. (2012). Tratamento das vulvovaginites na gravidez. *Femina*
- Stojanoski, N. (1999). Development of health culture in Veles and its region from the past to the end of the 20th century. *Veles: Society of science and art*, 13, 34.
- Subbiahdoss, G., Pidhatika, B., Coullerez, G., Charnley, M., Kuijer, R., van der Mei, H. C., ... & Busscher, H. J. (2010). Bacterial biofilm formation versus mammalian cell growth on titanium-based mono-and bi-functional coating. *Eur Cell Mater*, 19, 205-213.
- Sullivan, D. J., Westerneng, T. J., Haynes, K. A., & Coleman, D. C. (1995). *Candida dubliniensis* sp. nov.: phenotypic and molecular characterization of a novel species associated with oral candidosis in HIV-infected individuals. *Microbiology*, 141(7), 1507-1521.
- SUN, L. C., LI, S. Y., WANG, F. Z., & XIN, F. J. (2017). Research progresses in the synthetic biology of terpenoids. *Biotechnology Bulletin*, 33(1), 64.
- Swidsinski, A., Guschin, A., Tang, Q., Dörffel, Y., Verstraelen, H., Tertychnyy, A., ... & Jiang, X. (2019). Vulvovaginal candidiasis: histologic lesions are primarily polymicrobial and invasive and do not contain biofilms. *American journal of obstetrics and gynecology*, 220(1), 91-e1.

References

- Talapko, J., & Škrlec, I. (2020). The principles, mechanisms, and benefits of unconventional agents in the treatment of biofilm infection. *Pharmaceuticals*, 13(10), 299.
- Tan, B. H., Chakrabarti, A., Li, R. Y., Patel, A. K., Watcharananan, S. P., Liu, Z., ... & Riengchan, P. (2015). Incidence and species distribution of candidaemia in Asia: a laboratory-based surveillance study. *Clinical Microbiology and Infection*, 21(10), 946-953.
- Taniguchi M, Kataoka T, Suzuki H, Uramoto M, Ando M, Arao K, Magae J, Nishimura T, Ōtake N, Nagai K. Costunolide and dehydrocostus lactone as inhibitors of killing function of cytotoxic T lymphocytes. *Bioscience, biotechnology, and biochemistry*. 1995 Jan 1;59(11):2064-7.
- Tavanti, A., Davidson, A. D., Gow, N. A., Maiden, M. C., & Odds, F. C. (2005). *Candida orthopsilosis* and *Candida metapsilosis* spp. nov. to replace *Candida parapsilosis* groups II and III. *Journal of clinical microbiology*, 43(1), 284-292.
- Tohge, T., Watanabe, M., Hoefgen, R., & Fernie, A. R. (2013). The evolution of phenylpropanoid metabolism in the green lineage. *Critical reviews in biochemistry and molecular biology*, 48(2), 123-152.
- Treangen, T. J., & Salzberg, S. L. (2012). Repetitive DNA and next-generation sequencing: computational challenges and solutions. *Nature Reviews Genetics*, 13(1), 36-46.
- Treviño-Rangel, R. D. J., González, J. G., & González, G. M. (2013). Aspartyl proteinase, phospholipase, esterase and hemolysin activities of clinical isolates of the *Candida parapsilosis* species complex. *Sabouraudia*, 51(3), 331-335.
- Tsui, C., Kong, E. F., & Jabra-Rizk, M. A. (2016). Pathogenesis of *Candida albicans* biofilm. *Pathogens and disease*, 74(4).
- Tungmunnithum, D., Pinthong, D., & Hano, C. (2018). Flavonoids from *Nelumbo nucifera* Gaertn., a medicinal plant: Uses in traditional medicine, phytochemistry and pharmacological activities. *Medicines*, 5(4), 127.
- U.S. National Plant Germplasm System, *Dianthus caryophyllus* L. <https://npgsweb.ars-grin.gov/gringlobal/taxonomydetail.aspx?13826>.
- Ugbogu, O. C., & Akukwe, A. R. (2009). The antimicrobial effect of oils from *Pentaclethra macrophylla* Bent, *Chrysophyllum albidum* G. Don and

References

- Persea gratissima Gaerth F on some local clinical bacteria isolates. *African Journal of Biotechnology*, 8(2).
- Vallabhaneni S, Cleveland AA, Farley MM, Harrison LH, Schaffner W, Beldavs ZG, Derado G, Pham CD, Lockhart SR, Smith RM. Epidemiology and risk factors for echinocandin nonsusceptible *Candida glabrata* bloodstream infections: data from a large multisite population-based candidemia surveillance program, 2008–2014. *Open forum infectious diseases* 2015 1 (Vol. 2, No. 4).
 - Vanani, A. R., Mahdavinia, M., Kalantari, H., Khoshnood, S., & Shirani, M. (2019). Antifungal effect of the effect of *Securigera securidaca* L. vaginal gel on *Candida* species. *Current medical mycology*, 5(3), 31.
 - Verma, S., & Singh, S. P. (2008). Current and future status of herbal medicines. *Veterinary world*, 1(11), 347.
 - Walker, G. M., & White, N. A. (2017). Introduction to fungal physiology. *Fungi: biology and applications*, 1-35.
 - Wang, G., & Li, Y. (1985). Clinical application of safflower (*Carthamus tinctorius*). *Zhejiang. J Trad Chinese Med*, 20, 42-43.
 - Wang, H., Wang, Z., Liu, Z., Wang, K., & Xu, W. (2021). Membrane disruption of *Fusarium oxysporum* f. sp. *niveum* induced by myriocin from *Bacillus amyloliquefaciens* LZN01. *Microbial biotechnology*, 14(2), 517-534.
 - Wang, H., Wang, Z., Liu, Z., Wang, K., & Xu, W. (2021). Membrane disruption of *Fusarium oxysporum* f. sp. *niveum* induced by myriocin from *Bacillus amyloliquefaciens* LZN01. *Microbial biotechnology*, 14(2), 517-534.
 - Washington, J.A. (2012). *Laboratory procedures in clinical microbiology*. Springer Science & Business Media.
 - Weinstein, R. A., Gaynes, R., Edwards, J. R., & National Nosocomial Infections Surveillance System. (2005). Overview of nosocomial infections caused by gram-negative bacilli. *Clinical infectious diseases*, 41(6), 848-854.
 - Wiesenfeld, H. C., Hillier, S. L., Krohn, M. A., Landers, D. V., & Sweet, R. L. (2003). Bacterial vaginosis is a strong predictor of *Neisseria gonorrhoeae* and *Chlamydia trachomatis* infection. *Clinical Infectious Diseases*, 36(5), 663-668.

References

- World Health Organization. (2001). Global prevalence and incidence of selected curable sexually transmitted infections: overview and estimates.
- Wu, J. Y., Zhou, D. Y., Zhang, Y., Mi, F., & Xu, J. (2019). Analyses of the global multilocus genotypes of the human pathogenic yeast *Candida tropicalis*. *Frontiers in microbiology*, 10, 900.
- Wu, T., He, M., Zang, X., Zhou, Y., Qiu, T., Pan, S., & Xu, X. (2013). A structure–activity relationship study of flavonoids as inhibitors of *E. coli* by membrane interaction effect. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1828(11), 2751-2756.
- Xu, J. (2021). Is Natural population of *Candida tropicalis* sexual, parasexual, and/or asexual?. *Frontiers in Cellular and Infection Microbiology*, 11, 751676.
- Yaesh, S., Jamal, Q., Shah, A. J., & Gilani, A. H. (2010). Antihepatotoxic activity of *Saussurea lappa* extract on D-galactosamine and lipopolysaccharide-induced hepatitis in mice. *Phytotherapy research*, 24(S2), S229-S232.
- Yamahiro, A., Lau, K. H., Peaper, D. R., & Villanueva, M. (2016). Meningitis caused by *Candida dubliniensis* in a patient with cirrhosis: a case report and review of the literature. *Mycopathologia*, 181(7), 589-593.
- Yang, L. F., Liu, X., Lv, L. L., Ma, Z. M., Feng, X. C., & Ma, T. H. (2018). Dracorhodin perchlorate inhibits biofilm formation and virulence factors of *Candida albicans*. *Journal de mycologie medicale*, 28(1), 36-44.
- Yang, Y. L., Lin, C. C., Chang, T. P., Lauderdale, T. L., Chen, H. T., Lee, C. F., ... & Lo, H. J. (2012). Comparison of human and soil *Candida tropicalis* isolates with reduced susceptibility to fluconazole. *PloS one*, 7(4), e34609.
- Yazaki, K., Sasaki, K., & Tsurumaru, Y. (2009). Prenylation of aromatic compounds, a key diversification of plant secondary metabolites. *Phytochemistry*, 70(15-16), 1739-1745.
- Yigit, N., Aktas, E., Dagistan, S., & Ayyildiz, A. (2011). Investigating biofilm production, coagulase and hemolytic activity in *Candida* species isolated from denture stomatitis patients. *The Eurasian Journal of Medicine*, 43(1), 27.
- Zahara, K., Tabassum, S., Sabir, S., Arshad, M., Qureshi, R., Amjad, M. S., & Chaudhari, S. K. (2014). A review of therapeutic potential of *Saussurea*

References

- lappa-An endangered plant from Himalaya. Asian Pacific journal of tropical medicine, 7, S60-S69.
- Zhao, G., Zheng, X. W., Gai, Y., Chu, W. J., Qin, G. W., & Guo, L. H. (2009). Safflower extracts functionally regulate monoamine transporters. *Journal of ethnopharmacology*, 124(1), 116-124.
 - Zhou, F. R., Zhao, M. B., & Tu, P. F. (2009). Simultaneous determination of four nucleosides in *Carthamus tinctorius* L. and Safflower injection using highperformance liquid chromatography. *J. Chin. Pharm. Sci*, 18, 326-330.
 - Zhou, F. R., Zhao, M. B., & Tu, P. F. (2009). Simultaneous determination of four nucleosides in *Carthamus tinctorius* L. and Safflower injection using highperformance liquid chromatography. *J. Chin. Pharm. Sci*, 18, 326-330.
 - Zhou, F. R., Zhao, M. B., & Tu, P. F. (2009). Simultaneous determination of four nucleosides in *Carthamus tinctorius* L. and Safflower injection using highperformance liquid chromatography. *J. Chin. Pharm. Sci*, 18, 326-330.
 - Zuza-Alves, D.L.; de Medeiros, S.S.; de Souza, L.B.; Silva-Rocha, W.P.; Francisco, E.C.; de Araujo, M.C.; Lima-Neto, R.G.; Neves, R.P.; Melo, A.S.; Chaves, G.M. Evaluation of virulence factors in vitro, resistance to osmotic stress and antifungal susceptibility of *Candida tropicalis* isolated from the coastal environment of Northeast Brazil. *Front. Microbiol.* 2016, 7, 1783.



جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل / كلية العلوم للنبات

قسم علم الأحياء

**تأثير المستقلبات النباتية الثانوية المستخرجة من
بعض النباتات الطبية ضد أنواع الكانديدا
المعزولة من العينات السريرية في محافظة بابل**

رسالة قَدِّمتها

إلى مجلس كلية العلوم للنبات جامعة بابل، وهي جزء من متطلبات نيل درجة الماجستير
في علوم الحياة

الطالبة

زينب محمد كريم القريشي

إشراف

الاستاذ الدكتور

عبير فوزي الربيعي

1444 هـ

الاستاذ الدكتور

حسين جبر حسين

2022 م

الخلاصة

اجريت الدراسة الحالية في جامعة بابل -كلية العلوم للبنات -قسم علوم الحياة ، وكان الهدف من الدراسة عزل وتشخيص ومن ثم السيطرة على انواع الكانديدا المعزولة من عينات سريرية متمثلة بـ (المهبل و الفم) بواسطة استخدام مركبات الايض الثانوية متمثلة بـ (القلويدات و التربينويدات و الفلافونويد) ، المستخلصة من النبات الطبية (*Carthamus tinctorius* ، *Saussurea costus* ، *Dianthus caryophyllus L*) ، في هذه الدراسة تم تشخيص ثلاث انواع من الخمائر المرضية و هي (*Candida albicans* ، *Candida tropicalis* ، *Candida dubliniensis*) . كذلك تم اختبار قدرتها على انتاج عوامل الضراوة المتمثلة بـ (Hemolytic, Biofilm) و مقارنتها مع بعض المضادات الحياتية المتمثلة (*Fluconazole 150 mg* , *Ketoconazole 150 mg* , *Caspofungin 150 mg*) الفعالية التضادية الفطرية تم انجازها خارج الجسم الحي باستخدام طريقة انتشار الحفر داخل الاكار ضد انواع الكانديدا و ذلك بتحضير ثلاث تراكيز من كل مادة فعالة (100,50,25). ومقارنتها بعينة السيطرة الموجبة المتمثلة بالمضاد الحيوي فلوكونزول (*Fluconazole 150 mg*) وعينة السيطرة السالبة المتمثلة بـ (*Dimso 10%*) . اظهرت نتائج هذه الدراسة ان المركبات التربينية و الفلافونويد المستخلصة من براعم الزهرية لنبات القرنفل تفوقا معنويا تحت مستوى احتمالية $p < 0.05$ على المضاد الحيوي *Fluconazole* عند اختباره على انواع الكانديدا خصوصا بالتركيز *100 mg/ml* . كذلك اظهرت هذه الدراسة ان المركبات القلوية و الفلافونيدية و التربينية المستخلصة من جذور نبات القسط الهندي اختزال في نمو انواع الكانديدا تحت مستوى احتمال $p < 0.05$ و خصوصا بالتركيز *100 mg/ml* عند مقارنتها بعينة السيطرة السالبة . في نفس السياق اظهرت المركبات القلويدية و الفلافونويدية و التربينية المستخلصة من بذور نبات العصفر انخفاضا في نمو انواع الكانديدا تحت مستوى احتمالية < 0.05 و خصوصا عند التركيز *100 mg/ml* عند مقارنتها مع عينة السيطرة السالبة . ختاماً يمكن الاستنتاج ان مركبات الايض الثانوية مثل القلويدات و الفلافونيدات و التربينات المستخلصة من براعم الزهرية لنبات القرنفل و جذور نبات القسط الهندي و بذور نبات العصفر جدا فعالة في السيطرة على انواع الكانديدا المعزولة من عينات سريرية مختلفة مثل العينات المهبلية و الاسنان .