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Evaluation of the antibacterial activity of aqueous and alcoholic extracts of Monks pepper seeds on some pathogenic bacteria isolated from humans.

Research submitted to the Department of Biology, which is a
requirement for a Bachelor's degree

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ
الْحَكِيمُ)

صدق الله العلي العظيم

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Acknowledgements

- To a light that illuminates my darkness when days and circumstances extinguish me...
- To a cloud that shades me and waters me without wanting to return my beauty...
- To the hands that help me when I stumble, and push me to resist all these things that call for a fall.

To my familyTo my mum and dad.

- My production is nothing but your upbringing You gave me the pen, so thank you.

To My supervisor. Dr. Prof Huda Jasim Altameme

- What goes on in our heads is much more than what we say. What we say is much more than what we write. So I will stop. At the word Thanks from the bottom of my heart.

Roqia & Reyam

Abstract

This study evaluates the antimicrobial effects of aqueous and ethanolic extracts of *Vitex agnus castus* seeds (Lamiaceae family) against several microbial populations representing Gram-positive and negative bacteria such as *Escherichia coli*, *Enterococcus aerogenes*, *Staphylococcus sciuri*, *Citrobacter freundii*, *Aeromonas sobria* and *Serratia odorifera* was isolated from people suffering from urinary tract infection using standard agar disc diffusion technique.

Inhibition zones showed that *Enterococcus aerogenes* were resistant to all types of aqueous and alcoholic extracts of *V.agnus castus* seeds and to all concentrations used.

The current research suggests that the plant includes flavonoids, terpenoids, and steroids that may be useful in the creation of phytomedicine for the treatment of the bacterial illnesses that were examined. This investigation showed that an aqueous extract of *Vitex agnus castus* seeds had significant antibacterial activity against serious clinical infections.

1: INTRODUCTION

Since the beginning of civilization, the survival of the human race has depended on plants not only as a source of food and oxygen, but also as a source of natural remedies (Muthu *et al.*, 2010). Some wild and medicinal herbal plants contain chemical compounds of great importance that are by-products of the metabolism processes within the plant that are used for the purposes of perpetuating their lives or protecting and defending against other living organisms.

They can be called natural, by-products, or by-products and are often called active ingredients. According to the World Health Organization (WHO) Health Organization, more than 80% of the world's population depends on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemicals that produce a specific physiological effect on the human body, the most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins, and phenolic compounds (Chhetri *et al.* 2008).

Due to their simplicity of use and superior cost-effectiveness in comparison to pharmaceutical treatments produced by synthesis, medicinal wild plants and herbs have been valued throughout history as important aids in the management of deadly illnesses (Yeung *et al.*, 2020)

Plants have lately been employed for nutraceutical reasons since they are essential for the production of food and phyto-complexes with medicinal qualities that may be used to treat and prevent disease (Durazzo *et al.*, 2020; Lucarini *et al.*, 2020; Khasim *et al.*, 2020)

One of the Verbenaceae family comes back to (order Lamiales, class Magnoliopsida, and division Magnoliophyta) native to the Mediterranean region

and diffused in Europe, Asia, and North Africa (Rani and Sharma, 2013), *Vitex agnus-castus* L. (VAC) is a popular medicinal plant that has recognized beneficial effects on human health. However, according to the modern taxonomy of plants, it is classified as a member of the Lamiaceae family (Adamov *et al.*, 2022). It is a well-known plant all over the world and is referred to by a variety of names in various languages, such as Fruit de gattilier in French, Sauzgatillo in Spanish, Monchspfefferfrüchte in German, Panjangosht in Persian, Frutto di Agnocasto in Italian, Chaste tree and monk's pepper in English (Roemheld-Hamm, 2005). The name "agnus castus" comes from the Latin words "castitas" (chastity) and "agnus" (lamb); it is also known as the "chaste tree," which relates to its capacity to lessen sexual desire and to encourage chastity in both women and monks. Another frequent name for this spice is "monk's pepper," which comes from the fact that monks traditionally used it as a seasoning in their meals (Schulz *et al.*, 1999).

In addition to being able to be gathered in a number of different areas along riverbanks and seacoasts as a wild plant, it was able to be found naturally in the habitats of Southern Europe, the Mediterranean region, and Central Asia. (Ono *et al.*, 2011; Rani and Sharma, 2013). It is either a deciduous tree or a big shrub that has the potential to reach a height of between 1.5 and 2 m on average. The diameter of the leaves ranges from 7.6 to 10 cm, and they are finger-shaped, with 5–7 finger-like leaflets per leaf. They have a smell similar to pine and have a grayish-green appearance with a lighter underside (Rani and Sharma, 2013) The flowers are arranged in clusters and resemble long spikes of lavender flowers (Edward and Watson, 1993). They range in color from violet to blue to deep purple and have a pleasant scent. The flowers bloom from the middle of summer until the beginning of fall, and the fruits are purple blackberries that carry four seeds that are similar to black pepper (Mancho and Edwards, 2005). (see to Figure 1)



Figure 1: dry and fresh of vegetative parts of *V.agnus castus*

Since both the fruit berries and the dried leaves of this plant have been used for purposes related to medicine, the plant in question is categorized as a herbal product (Mancho and Edwards, 2005). The fruit of the *V.agnus castus* is the component of the plant that is used medicinally (Girman *et al.*, 2003; Mari *et al.*, 2015). Research has shown that this plant has numerous therapeutic effects, including antioxidant, chemo preventive, immunomodulatory, and cytotoxic; tumoricidal; antimutagenic; antimicrobial; antifungal; insect repellent; larvicidal; fracture healing; osteopenic; anti nociceptive; opioidergic; antiepileptic; and anti-inflammatory properties. The negative reactions to this plant's use are temporary and minor (Niroumand *et al.*, 2018).

Phytotherapists have long utilized *V.agnus castus* to treat a wide range of women's health issues, including but not limited to menstruation problems (amenorrhea, dysmenorrhea), PMS, infertility, acne, menopause, and

disruptions in breastfeeding and the production of prolactin (the hormone responsible for lactation) (Christie and Walker, 1997). The fruits of *Vitex agnus-castus* have long been used medicinally, particularly for boosting milk volume, relieving gas, and stopping diarrhea (Niroumand *et al.*, 2018).

There have been a lot of studies done on the different health-promoting potentials of this plant, including its antioxidant, immunomodulatory, cytotoxic, antimutagenic, antimicrobial, antifungal, antinociceptive, opioidergic, antiepileptic, and anti-inflammatory properties, as well as its benefits for osteopenic syndromes. There have also been a lot of reported data on these different health-promoting potentials (Al Saka *et al.*, 2017; Heskes *et al.*, 2018).

2: MATERIALS AND METHODS:

2-1: Collection of Plant Material:

Vitex agnus castus seeds were obtained from the local markets of Hilla City (Figure 2), which were identified according to a morphological characteristic in the flora of Iraq (Townsend *et al.*, 1974). Samples were transported to the home for cleaning by washing with tap water to remove dust and insects, then pounded by pestle and mortar lastly sieved to get the powder of plant materials to be prepared for extraction.



Figure 2: seeds of *V.agnus castus* collected from local market

2-2: Preparation of the Extracts of aqueous water for Plant

The hot boiled and cold water extracts were prepared for the above plant parts according to Harborne's maceration method (1998).

- 1- Macerate 100 g of plant material in a beaker with 1000 ml hot boiled and cold water, agitated for 30 minutes
- 2- After leaving for 24 hours to decompose the active ingredients more effectively, cover them tightly to prevent any foreign materials from entering.

- 3- Filter the solutions through two layers of gauze and centrifuge them for 10 minutes at 3000 rpm.
- 4- Take the leachate and leave the residue, then place the solution in (40-45) C° an electric oven to acquire a dry material from the extract.
- 5- Keep refrigerated in glass containers until ready to use. The process was repeated numerous times to obtain a significant amount of the extract to conduct experiments.
- 6- Prepare the stock solution concentration equivalent to 300 mg/ml after 7.5 gm of the crude plant was dissolved in 25 ml of distilled water. The concentrations (200,100) mg/ml were produced from this solution using the formula $N1V1 = N2V2$ (Al-Nakeeb, 2004, Awwad *et al.*, 2014).

2-3: Preparation of the Extracts of alcohol for Plant

All steps mentioned above in the aqueous extract are reapplied using ethanol at a concentration of 70% (Figure 3).



Figure 3: Alcohol and aqueous extracts of *V.agnus castus* seeds

2-4: Antibacterial Activity: The anti-bacterial activity of the bioactive compounds extracted from the *Vitex agnus castus* seeds was tested against the isolated bacteria by using agar-well diffusion method, Measurement of diameters of clear inhibition zones was recorded as antibacterial activity (Perez *et al.*, 1990).

2-5: Microorganism Strain: *Escherichia coli*, *Enterococcus aerogenes*, *Staphylococcus sciuri*, *Citrobacter freundii*, *Aeromonas sobria*, *Serratia odorifera* were isolated from people suffering from urinary tract infection.

3: RESULTS AND DISCUSSION:

Three types of extracts were prepared from the seeds of the *V.agnus castus* which are hot water extract, cold water extract, and ethanolic extract. Different concentrations of these extracts (300, 200, 100 mg/ml) were used to detect their inhibitory effect on different types of bacteria (*Enterococcus aerogenes*, *Aeromonas sobria*, *Escherichia coli*, *Staphylococcus sciuri*, *Citrobacter freundii* and *Serratia odorifera*). The results of the study of the inhibitory effectiveness varied according to the type of extract and the different types of bacteria, and there was an apparent increase in the rate of the diameter of the inhibition zones by increasing the concentration of each of the plant extracts towards the growth of bacteria, as the diameters of the zones of inhibition of the hot aqueous extract towards the growth of bacteria ranged between 12-15mm and cold water from 12-17mm and for the ethanolic extract 12-20mm. Through the rates of the diameters of the zones of inhibition it became clear that the aqueous ethanol extract had clear effectiveness as it recorded the highest rates of inhibition reached 20 mm for *E.coli* bacteria at a concentration of 300, mg/ml and 17 mm for the cold water extract in *Aeromonas* bacteria as for the alcoholic extract the highest inhibition zone of 15 mm was recorded in both *Staphylococcus sciuri* and *E. coli* (Table 1).

The results also showed that the bacteria, *Enterococcus aerogenes*, were resistant to all types of aqueous and alcoholic extracts of *V.agnus castus* seeds and to all concentrations used.

The effectiveness of the alcoholic extract is less than the aqueous extract, and this is consistent with the researchers, and the reason is due to the ability of the active substances to dissolve in water at a higher rate than in alcohol, which increases the effectiveness of the aqueous extract.

Table 1: Antimicrobial activity of examined plant extracts on six microorganism using disk diffusion method

Bacteria Pathogen	Alcohol and aqueous extracts of <i>V.agnus castus</i>								
	Hot water			Cold water			Ethanol		
	Concentration mg /ml								
	300	200	100	300	200	100	300	200	100
	Inhibition zone / mm								
<i>Staphylococcus sciuri</i>	15	12	R	R	R	R	18	13	R
<i>Enterococcus aerogenes</i>	R	R	R	R	R	R	R	R	R
<i>Citrobacter freundii</i>	R	R	R	16	13	R	R	R	19
<i>Aeromonas</i>	R	R	R	17	14	12	R	R	R
<i>Escherichia coli</i>	15	R	R	R	R	R	20	13	12
<i>Serratia odorifera</i>	13	R	R	15	12	R	R	R	R

The inhibitory effects of the plant leaf extract are due to the presence of flavones, which are known for their ability to rupture cellular membranes by forming complexes with the extraneous proteins present in them. Also, phenols increase the activation of enzymes responsible for basic metabolic reactions through their specialized interference with proteins, which leads to their deformation. (Protein denaturation) and then the inability of the bacteria to continue to grow as the alkaloids interfere with the DNA of the bacteria cells and inhibit their growth. The reason may be due to the nature of the outer membrane and its different permeability.

The aqueous extracts were the most effective in inhibiting the growth of the tested bacteria, followed by the alcoholic extract. The microorganisms most affected by the aforementioned extracts were the Gram-positive bacteria represented by *Staphylococcus sciuri*, then the Gram-negative Bacteria *E. coli*, *Aeruginosa*, and the least Gram-negative bacteria affected by the extracts were *Enterococcus aerogenes*.

The aqueous extract of the plant parts used in the study led to a significant inhibition of the growth of all types of bacteria pathogenic to humans under study, with the exception of *Enterococcus aerogenes*, and this is due to the fact that these extracts contain substances that have the ability to inhibit the growth of some microorganisms, and this is consistent with previous studies, which indicated that such this inhibition is due to the presence of glycosides as well as the effect of other effective groups such as alkaloids, resins, phenolic compounds, tannins and essential oils, which made the aqueous extract effective against experimental organisms, and the difference in the effect of these secondary metabolites in the effect is due to the different types and quantities of these active substances.

Alkaloids, polyphenols, flavonoids, anthraquinones, coumarins, saponins, tannins, triterpenes, and steroids were found in the phytochemical screening. Pathogenic bacteria use many compounds (Erfan and Marouf, 2019). Metabolites in the examined plant extracts may explain their antimicrobial and antibacterial properties. Extracts have different antibacterial properties. They may vary in chemical makeup and bioactive ingredient mode of action (Cowan, 1999). All plant extracts contain secondary metabolites, but their activity depends on their concentration and possible interactions with other components (Dzotam *et al.*, 2016).

Hoberg *et al.* (2000) reported finding flavonoids, tannins, iridoids, and diterpenoids in the fruits, flowers, and leaves of *Vitex agnus-castus*.

Sağlam *et al.*, (2007) phytochemical investigation of *V.agnus-castus* revealed the presence of flavonoids and tannins in the plant's fruits and leaves. Polyphenols, flavonoids, and tannins have all been shown to have antioxidant properties (Van Acker *et al.*, 2000).

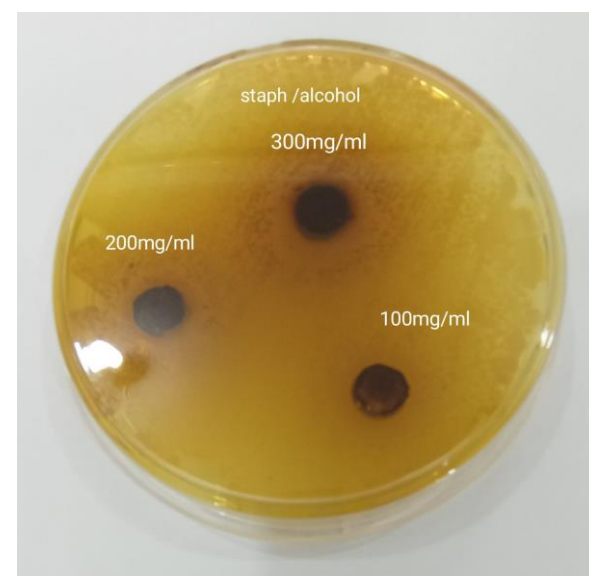
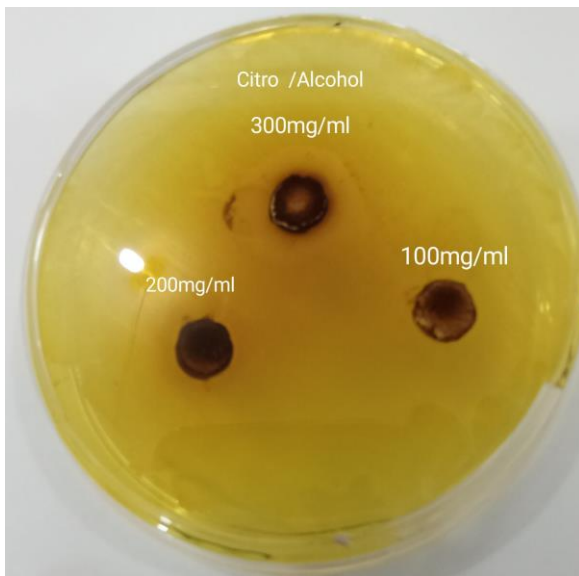
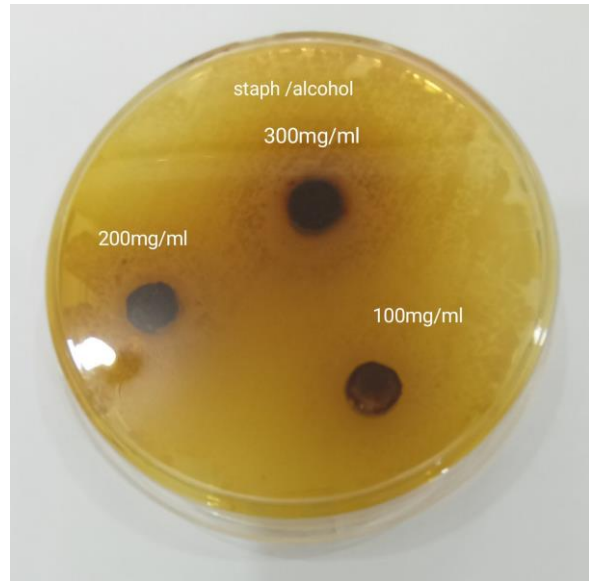
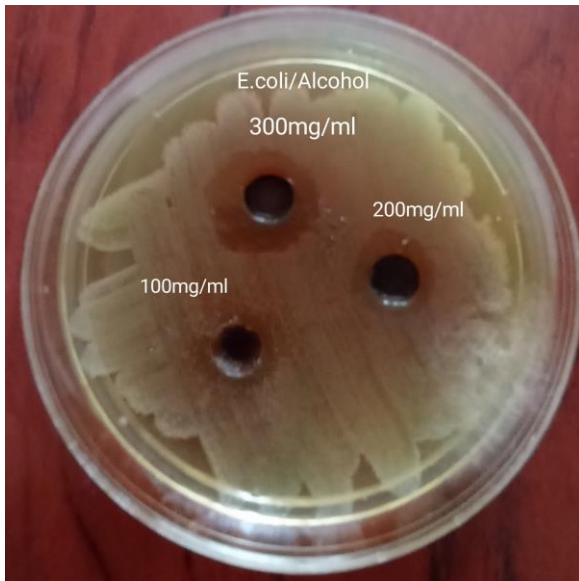


Figure 4: Antimicrobial activity of *V.agnus castus* seeds extract on some microorganism using disk diffusion method

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