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ANTIBACTERIAL EFFECT of Aqueous Extracts for (Olibanum, CARDAMOM, and Clove) on *Proteus Mirabilis* Isolated from Urinary Tract Infections

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ABSTRACT

Background: Proteus mirabilis is a bacterium of medical importance in infections of urinary sewers and is a major cause of associated diseases such as kidney stones and others. It is also resistant to many antibiotics, so the study resorted to the use of natural substances to study their effectiveness towards these bacteria, **Aim of study** The study aimed to determine the effect of aqueous extracts for (olibanum, cardamom, and clove) on Proteus Mirabilis isolated from urinary tract infections for the purpose of usng it as therapeutic alternatives.

Methodology: Bacteria were collected from patients with urinary sewage inflammation and diagnosed by chemical and microscopic methods. They then attended water extracts of these substances and various concentrations (10, 20, 50, 100 g/mL). The bacteria were planted in the middle of the Mueller Hunton Acar and treated with these extracts to study the areas of inhibition.

Results: The highest incidence among males aged 40-70 was 22% of the total samples, and the effect of olibanum was most on the 15-40 age group in males, while the least influential on this group was cardamom, while the 40-70 age group in males clove was most influential in its different concentrations while cardamom was least, the synergistic and antagonistic effectiveness of all extracts as the synergistic effectiveness was at its highest level when the extracts (cardamom+clove) were combined.

Conclusion: Combining cardamom, and clove extracts with each other has had a high synergistic effect and therefore the use of these substances with each other by humans may reduce the incidence of urinary tract infections caused by proteus mirabilis.

1.Introduction

1.1.Proteus bacteria

Enterobacteriaceae is a family of gram-negative bacteria that includes Proteus species. They are lactose-intolerant facultative anaerobes that can ferment maltose but not lactose. Hauser documented and described the first isolates in the late 19th century. Proteus mirabilis, Proteus vulgaris, Proteus penneri, Proteus hauseri, Proteus terrae, and Proteus cibarius are the species that now make up the genus(1). The majority of clinical Proteus isolates come from P mirabilis and P vulgaris. Proteus organisms, along with Escherichia, Klebsiella, Enterobacter, and Serratia species, are thought to be important causes of infections in humans

Along with Escherichia coli and Klebsiella species, of which E. coli is the predominant inhabitant, Proteus species are most frequently found in the human digestive system as part of typical human intestinal flora. Proteus can also be found in a variety of environments, including hospitals and long-term care homes. Gram-negative bacilli frequently invade the skin and oral mucosa of patients and hospital

staff in healthcare facilities. These reservoirs are primarily where infection occurs. Proteus species do not, however, cause the majority of nosocomial infections. (2)

Ninety percent of Proteus infections are brought on by P mirabilis, which is regarded as a communitarian infection. P vulgaris and P penneri may be isolated from people living in nursing homes and hospitals, as well as from patients who have underlying illnesses or weakened immune systems .(3)

There is an increased risk of infection from Proteus and other organisms (such as Klebsiella, Enterobacter, Pseudomonas, enterococci, and staphylococci) in patients with recurrent infections, structural abnormalities of the urinary tract, urethral instrumentation, and infections acquired in the hospital. (4)

1.1.1.Pathology

The extracytoplasmic outer membrane of Proteus species is a characteristic of gram-negative bacteria. A lipid bilayer, lipoproteins, polysaccharides, and lipopolysaccharides are other components of the outer membrane .(5)

The interplay of the infectious organism and the host's defensive mechanisms determines whether an infection occurs. The host and several membrane elements interact to determine pathogenicity. The size of the inoculum is significant and positively correlated with the likelihood of infection. (6)

There are some bacteria-specific virulence factors known. The adhesion of the bacterium to the host tissue is the initial stage of the infectious process. Fimbriae promote adhesion, which increases the organism's ability to cause disease. Fimbriae, or pili, are small projections on the surface of gram-negative bacteria including E. coli, P mirabilis, and others. Organisms can adhere to certain host tissue areas, such as the endothelium of the urinary tract, thanks to specific molecules that are situated on the ends of the pili. It has been established that the presence of these fimbriae is crucial for P mirabilis' adhesion to host tissue. (5)

1.2.Urinary tract infections (UTIs)

Especially in women, infants, and the elderly, urinary tract infections (UTIs) are a highly prevalent condition. In their lifetime, one in every two women and one in every 20 males will develop a UTI.(7)

The kidneys regulate the blood's water content and remove waste materials to produce urine. The ureter, which connects each kidney to the bladder, is a tube. Through the ureters and into the bladder, urine exits the kidneys. Urine exits the body through a tube known as the urethra once the bladder "signals" the need to urinate. To reduce the possibility of a major kidney infection, the urinary system was created. By stopping the urine from the bladder from rising back up into the kidneys, it accomplishes this. Although they cause symptoms, most urinary infections are rarely serious or life-threatening since they are limited to the bladder. (8)

1.2.1.What causes UTIs?

Typically, bacteria do not reside in the urinary system. A UTI can be brought on by germs that enter the urinary tract and grow there (9).

Cystitis or urinary infections can be brought on by a wide variety of microorganisms. Escherichia coli (E. coli), the most frequent germ responsible for urinary tract infections, is located in your digestive system. E. coli can easily adhere to the lining of your urinary tract and spread to the urethra .(10)

Ureteritis can be brought on by bacteria like Chlamydia and Mycoplasma in both men and women. Both couples must receive medical care to prevent re-infection because these bacteria can be transmitted during sexual activity(8).

1.2.2.Avoidance of UTIs

Despite not necessarily being supported by clinical evidence, some women have found the following advice to help lower their chance of having urinary tract infections, to flush the urinary system, consume a lot of water and other liquids, get rid of trichomonas or other vaginal infections right away, use caution when utilizing spermicide-containing products, especially when using a diaphragm contraceptive .(9)

As soon as you have the desire to urinate, immediately go to the bathroom rather than holding it in, after using the restroom, wipe yourself from front to rear (urethra to anus), to prevent constipation (8).

In the past, cranberries have been used to stop UTIs (often in cranberry juice form). A compound in cranberries can stop the E. coli bacteria from adhering to the cells lining the urinary tract. However, recent studies have revealed that cranberry juice does not significantly reduce the risk of UTIs, and the majority of people find it difficult to consume the juice consistently over an extended period. If you plan to drink cranberry juice, let your doctor know because it can reduce the effectiveness of some antibiotics. (10)

1.3.Olibanum (Boswellia sacra)

A little tree endemic to southern Saudi Arabia and North Africa called Boswellia, olibanum is a natural gum resin that develops when incisions are made in the trunks of several varieties. In the wild, local communities frequently harvest boswellia. A few weeks later, the

gum is collected. The varied quality is then sorted for grading and cleaning to get them ready for shipment. Biolandes uses wild Boswellia sacra gum collected in Salalah, the "perfume capital of Arabia," in southern Oman, as well as Boswellia carterii gum collected in designated areas of northern Somalia to ensure a supply of extracts of consistent analytical quality. To provide organic essential oils, Biolandes has organized a dedicated supply of organic olibanum gum during the past few years. (11)

1.4.Cardamom (Elettaria cardamomum)

A perennial herb called cardamom (Elettaria cardamomum) is indigenous to portions of Asia, including Bangladesh, Pakistan, and Indonesia. It belongs to the Zingiberaceae plant family, which also contains ginger, and has white blossoms.

Numerous Asian dishes, such as coffee, pastries, and curries, frequently include dried seeds as a seasoning. For their possible medicinal properties, several parts of the cardamom plant, including the seeds, leaves, bark, roots, and flowers, have been utilized for thousands of years. (12)

Cardamom has been found to have antioxidant, anti-inflammatory, antihypertensive, cholesterol-lowering, and blood sugar-lowering properties in preliminary research trials.3 Clinical studies demonstrating these and other health advantages, however, are scarce. (12)

1.5.Clove (Syzygium aromaticum)

Clove is a spice made from the tiny, reddish-brown flower buds of the tropical, evergreen tree Syzygium aromaticum, which belongs to the Myrtaceae family. Cloves are said to be native to the Indonesian Moluccas, sometimes known as the Spice Islands, and were a significant component of the first spice trade. Cloves are used to flavor a wide variety of meals, especially meats and bakery goods. In Europe and the United States, the spice is a distinctive flavoring in Christmas holiday fare like wassail and mincemeat. (13)

Aim of study The study aimed to determine the effect of aqueous extracts for (olibanum, cardamom, and clove) on Proteus Mirabilis isolated from urinary tract infections for the purpose of usng it as therapeutic alternatives

2.Materials and methods

2.1.Samples collection

The samples of a group of patients with urinary tract infections (males and females) at different ages (15 to 70 years) were collected at time from 1/3/2023 to 1/7/2023 from many hospitals of Babylon governorate, Iraq , to detect and isolate infections of proteus mirabilis for study, the number of these samples was 200.

2.2.Diagnosis of Proteus mirabilis

Samples conducted a series of biochemical and microscopic tests to diagnose proteus mirabilis bacteria.

2.3.Preparation of aqueous extracts

Washed 100 g of both olibanum, cardamom, and clove with distilled sterile water three times, then grind well, add 100 ml of distilled sterile water, and soak well for 24 hours under sterile conditions, then drain the water only to be concentrated 100:100 g/ml and then extract the other concentrations according to the equation C1V1 = C2V2. To prepare four concentrations for all material (100 mg/ml, 50 mg/ml, 20mg/ml, 10mg/ml)

2.4. Activity assay

Media of Müller Hunton Agar was prepared and the bacterium proteus mirabilis was cultured on it. Then a 10-mm well was made by the cork borer. The extracts were placed in different concentrations in these wells and incubated for less than 24 hours at a temperature of 37m. Distilled sterile water was used as a control.

2.5. Synergistic activity assay

We are mix two from three materials at every time with minimum concentration to determine synergistic activity with concentraton (10 mg/ml).

2.6. Statistical analysis

Used special package for statistical sciences SPSS version 23 to analysis our results, one way ANOVA used for this purpose.

3.Results and Discussion

3.1. Biochemical and microscopical tests

Table 1: Biochemical and microscopical tests

test	indole	VP	MR	motility	urease	catalase	citrate	Gelatin hydro-lase	Gram stain
result	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve

3.2. Distribution of samples

Table 2: Distribution of samples

samples	No. of samples	Positive samples	percentage
Male 15-40 years	50	3	6%
Male 40-70 years	50	11	22%
Female 15-40 years	50	9	18%
Female 40-70 years	50	8	16%

Table 3 effect of olibanum aqueous extract at different concentration on proteus

Through Table 3, the highest concentration was the highest inhibition, 100 mg/mL, while the lowest concentration was the lowest inhibition and effectiveness, but it was also effective in inhibiting bacterial growth and there were morally significant statistical differences at 0.05.

Table 4 effect of cardamom aqueous extract at different concentration on proteus

Material concentration (mg/ml)	Inhibition zone (mm)	Material concentration (mg/ml)	Inhibition zone (mm)
olibanum 100	22	olibanum 50	22
olibanum 100	23	olibanum 50	21
olibanum 100	32	olibanum 50	20
olibanum 100	31	olibanum 50	19
olibanum 100	29	olibanum 50	17
olibanum 20	15	olibanum 10	9
olibanum 20	17	olibanum 10	11
olibanum 20	14	olibanum 10	6
olibanum 20	13	olibanum 10	8
olibanum 20	16	olibanum 10	0
All groups are significance differences with each other at P≥ 0.05			
Material concentration (mg/ml)	Inhibition zone (mm)	Material concentration (mg/ml)	Inhibition zone (mm)
cardamom 100	22	cardamom 50	21
cardamom 100	32	cardamom 50	22
cardamom 100	36	cardamom 50	19
cardamom 100	33	cardamom 50	25
cardamom 100	29	cardamom 50	18
cardamom 20	13	cardamom 10	8
cardamom 20	15	cardamom 10	6
cardamom 20	16	cardamom 10	6
cardamom 20	12	cardamom 10	9
cardamom 20	9	cardamom 10	6
All groups are significance differences with each other at P≥ 0.05			

Table 5 effect of clove aqueous extract at different concentration on proteus

Material concentration (mg/ml)	Inhibition zone (mm)	Material concentration (mg/ml)	Inhibition zone (mm)
clove 100	33	clove 50	22
clove 100	37	clove 50	25
clove 100	32	clove 50	21
clove 100	28	clove 50	20
clove 100	30	clove 50	28
clove 20	13	clove 10	7
clove 20	15	clove 10	6
clove 20	13	clove 10	0

clove 20	16	clove 10	5
clove 20	9	clove 10	8
All groups are significance differences with each other at P≥ 0.05			

Through Table 4 and 5 we note that all the concentrations used were effective against bacteria and their effectiveness was gradually higher to lower and there were morally significant statistical differences at 0.05.

Table 6 effect of mix aqueous extracts at concentration (10 mg/ml) on proteus

Material concentration (mg/ml)	Inhibition zone (mm)	Material concentration (mg/ml)	Inhi-bi-tion zone (mm)
olibanum +cardamom	12	Olibanum +clove	15
olibanum +cardamom	14	Olibanum +clove	8
olibanum +cardamom	9	Olibanum +clove	12
olibanum +cardamom	11	Olibanum +clove	14
olibanum +cardamom	13	Olibanum +clove	14
Cardamom +clove	17		
Cardamom +clove	11		
Cardamom +clove	10		
Cardamom +clove	12		
Cardamom +clove	9		
All groups are significance differences with each other at P≥ 0.05			

Through Table 6 where the combination of two materials is used to measure the lasting effectiveness of the substances used and through the lowest user concentration of 10 mg/ml per substance, we found that the effect of cardamom + clove is the highest effect indicating the synthetic effectiveness of these two substances to work together against bacteria and is higher than their effectiveness at the concentration of 20 mg/ml individually in tables 4 and 5.

While olibanum + clove was less effective, indicating that one was unimpressed by the other, their individual effectiveness at this focus was an approach to their combined effectiveness.

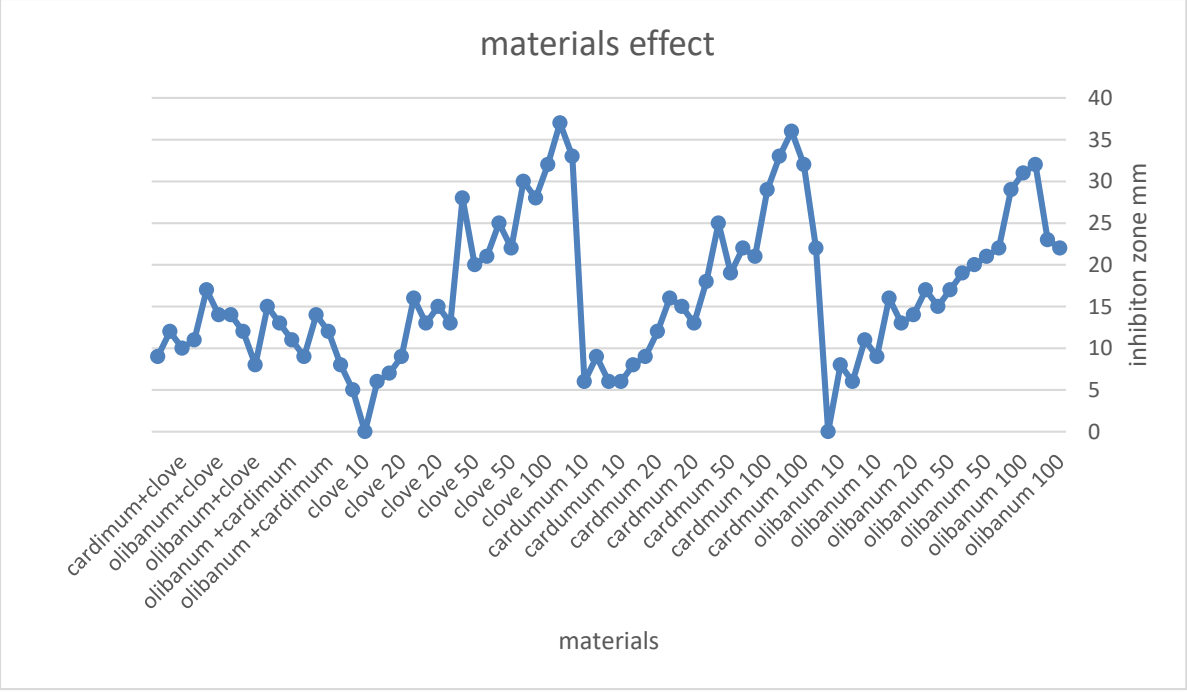

Figure 1 effect of different materials at different concentrations on proteus

Conclusion

Combining cardamom, and clove extracts with each other has had a high synergistic effect and therefore the use of these substances with each other by humans may reduce the incidence of urinary tract infections caused by proteus mirabilis.

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