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Antimicrobial Activity of Aqueous Methanolic Extract of Lichen *Usnea barbata* Against *E. coli* and *S. aureus*

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ABSTRACT

In this present study, various concentrations (0.5, 1, and 2%) Aqueous Methanolic Extract of Lichen (Usnea barbata) was tested in vitro for their antibacterial activities against two pathogenic bacteria Escherichia coli and Staphylococcus aureus, using the disk diffusion method. On the other hand, Erythromycin and Florfenico were used as a positive control while the negative control was cotton oil. In this study, there are no antibacterial effects of the Extract of Lichen (Usnea barbata) used in this study.

النشاط المضاد للميكروبات للمستخلص الميثانولي (Usnea barbata) ضد الإشريكية القولونية Escherichia coli والمكورات العنقودية الذهبية Staphylococcus aureus .

محمد عمر عبدالله سالم

في هذه الدراسة، تم اختبار تركيزات مختلفة (0.5، 1، و 2٪) من المستخلص الميثانولي لـ (*Usnea barbata*) في المختبر كمضاد للبكتيريا ضد اثنين من البكتيريا المسببة للأمراض هما الإشريكية القولونية Escherichia coli والمكورات العنقودية الذهبية Staphylococcus aureus، وذلك باستخدام طريقة انتشار القرص. من ناحية أخرى، تم استخدام الاريثروميسين والفلورفينيكول كسيطرة موجبة بينما كانت السيطرة السلبية لزيت القطن. أشارن النتائج بأنه لا توجد أي تأثيرات مضادة للجراثيم لمستخلص (Usnea barbata) المستخدم في هذه الدراسة.

INTRODUCTION

The current scientific community incorporates a tall degree of mindfulness, which has made a difference to progress investigate on normal items and increment information around the association between a compound's natural properties and its chemical structure (Salem et al., 2021; and Cheng et al., 2009). Since they are accepted to have negligible natural impacts and can be utilized as natural control operators instead of anti-microbials to oversee microbes that have ended up safe, characteristic plant items have long been utilized as an elective source of antimicrobials (Salem, 2022). For these reasons, normal sources are imperative materials for the think about of their organically strong applications as secure sources that work as novel antibacterial specialists of different bunches of microorganisms (Salem et al., 2023).

Around the world, a assortment of sicknesses are treated with therapeutic and pharmaceutical plants and plant subsidiaries (counting entirety plants, fundamental oils, extricates, powder, and photochemical) (سالم،2023; Phillipson, 1994). More than 21,000 plants are utilized remedially around the world, concurring to WHO information from 1996 (Noor et al., 2013). In expansion to being cheap to deliver, plant items have numerous

benefits for illness control, such as being biodegradable, effectively available, and competent of battling pathogenic microscopic organisms without posturing any natural dangers or negative side impacts (Salem et al., 2022 and Rayet al., 2004).

Due to their capacity to stifle microbes, counting both Gram-positive and Gram-negative bacteria, plant derivatives' antibacterial action has been altogether examined against a run of organisms (Taştan& Salem, 2021). Be that as it may, the appearance of microscopic organisms that are safe to a few drugs presents a trouble for the treatment of bacterial infections, emphasizing the need of finding novel antimicrobial substances to combat these infections (Hemaiswaryaet al., 2008).

So, the study aimed to investigate the antibacterial activity of Aqueous Methanolic Extract of Lichen (Usnea barbata) against tow pathogenic bacteria Staphylococcus aureus, Escherichia coli. Moreover, the antimicrobial activities of Lichen were compared with erythromycin and fluorfenicol.

MATERIALS AND METHODS

PREPARATION OF LICHEN (*Usnea barbata*) EXTRICATE

The ready lichen was extricated using a methanol extraction strategy as depicted by Bilen et al (2020), with some adjustments:

Lichen was ground into a fine powder using a mechanical processor, and 50 g of this powder was included to 1 L of 40% methanol. The blend was at that point cleared out to stand at room temperature for three days whereas being shaken every day. After three days, the lichen extricate was sifted through filter paper, collected, and vanished at 55 to 65 degrees Celsius in a rotating evaporator to evacuate the liquor. The wrapped up item, which taken after nectar, was broken up in refined water to form different concentrations (0.5, 1, and 2%) and put away at 4 ° C.

BACTERIAL PREPARATION

Gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) pathogenic bacteria were pre-cultured in nutrient broth for a full night at 37°C in a rotary shaker. The bacteria were then centrifuged at 10,000 rpm for six minutes, and the pellet was suspended in double-distilled water (adjusted to the 0.5 McFarland standards). Antimicrobial activity tests were conducted against these bacteria.

RESULTS AND DISSCUSION

According to Bauer et al. (1966), the technique of disc diffusion was utilized for antimicrobial testing in order to ascertain whether Extract possessed antibacterial qualities. The Petri plates containing the sterile Salmonella Shigella (SS) Agar and Brain Heart Infusion B.H.I Agar were filled and left to solidify. Using a sterile Glass spreader ,the bacterial suspension was spread throughout the agar when the turbidity of the bacterial colonies was brought to the 0.5 McFarland standard (1.0x108 CFU/mL).

The antibacterial activity of the plates was examined after they had dried for fifteen minutes. Disc Dispenser was used to place the discs on the agar surface, and they were subsequently impregnated with 20 L of mother concentrated extract solution and diluted concentration as mentioned above.

Several disks make up each plate. Positive controls included treated discs, a negative control, and an ordinary commercial antibiotic disk. Standard antibiotic discs containing 15g/mL of erythromycin and 30g/mL of fluorfenicol were used as a positive control. The negative control of cotton oil.

After that, the plate was incubated at 37° C for 24 hours. The plates were incubated, and then the inhibitory zone was examined. Then, calipers were used to measure and record the inhibition zone. The tests were conducted twice to ensure reliability.
It was found that the *E. Coli* inhibition zone measured between 11.00 and 13.90 mm at 0.5%. At 1% and 2% concentrations, the inhibition zone's diameter was found to be between 9.00 and 10.90 mm and 7.00 and 8.90 mm, respectively (Figure 1).

In all plant seed oil concentrations, the diameter of the inhibitory zone in *S. aureus* was found to be between 7.00 and 10 mm.

Using cotton oil as a negative control, antibacterial activities against *S. aureus* and *E. coli* were not seen. In order to investigate the antibacterial activity of Lichen (*Usnea barbata*) Extract at various doses, erythromycin (15g/mL) and fluorfenicol (30g/mL) were used as the study's antibiotics. In the disk diffusion assays, both antibiotics showed an antibacterial effect on the two pathogens.

Figure 1. Antimicrobial activity of Lichen (*Usnea barbata*) Extract against *Escherichia coli* using disc diffusion method.

DISCUSSION

The impacts of different concentrations (0.5%, 1%, and 2%) of lichen (*Usnea barbata*) extricate utilized as an *antibacterial against pathogenic microbes were examined within the current ponder.*

The comes about detailed in figures (1,2 and 3) appeared the antibacterial movement of the *Usnea barbata* extricate against *S. aureus* and *E. coli*. The Lichen (*Usnea barbata*) Extricate tried for antibacterial movement at distinctive dosages and appeared a few impacts against *E. coli* with an hindrance zone between 9.00 and 13.90 mm at the 0.5% concentration Not at all like the 2% concentration, which had an inhibitory zone distance across of 10.00 mm and no bactericidal action, has this moreover applied to the 0.5% concentration.

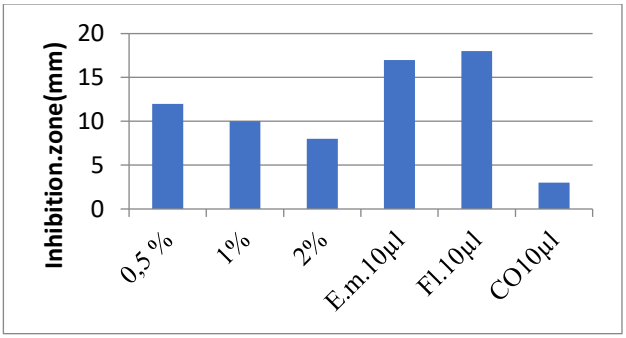

Figure 2. Antimicrobial activity of Lichen (*Usnea barbata*) Extract against *Escherichia coli* using disc diffusion method.

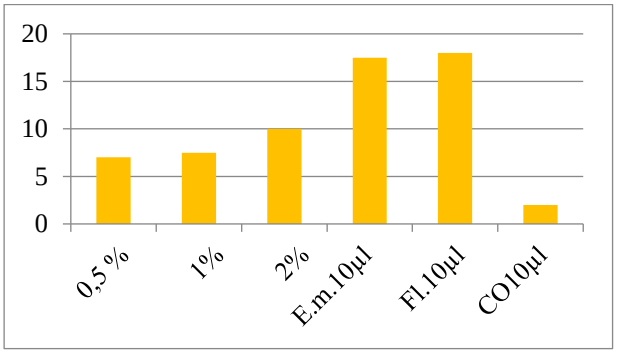

Figure 3. Antimicrobial activity of Lichen (*Usnea barbata*) Extract against *Staphylococcus aureus* using disc diffusion method

Typically most likely since the alcoholic division is thought to contain the almost of the chemical components with antibacterial Activities (Popoviciet al., 2022). This was reliable with the discoveries of Ahmad et al (2024), who found that liquor outflanked water and hexan as a solvent for the extraction of antimicrobial dynamic ingredients. The extraction handle may be the cause of this dissimilarity. It is still hazy precisely which dynamic phytocompounds in *Usnea barbata* display antibacterial activity. In any case, it is accepted that the most component mindful for the antibacterial properties is usnicacid (Cocchiettoal., 2002).

Numerous agents inspected *U. barbata* and *Usnea* sp. for antibacterial movement; in common, their discoveries coordinated our study's (Cansaranet al., 2006).

The affect of *U. barbata* extricates in methanol and ethyl-acetate against 13 distinguished Staphylococcus sp. ensnared in bovine mastitis was inspected by Idamokoro et al. 2014. They illustrated that methanol extricates had the next inhibitory adequacy than ethyl-acetate extricates.(Mesta et al. 2020) detailed that the ethanol extricate of *U. ghatensis* (15 mg/mL) against *S. aureus* measured 12 mm, while the ethanol extricate of *U. undullata* (15 mg/mL) on *S. pneumonia* appeared 18 mm. In an prior investigate. Noteworthy viability against *S. aureus* was too illustrated by *U. florida* extricate in methanol.

The conditions for extricating *U. subfloridana* were optimized by Boisova et al. 2020, (for 80 min, at a temperature of 85 ° C and weight of 150 atm). The extricate they got had solid antibacterial movement against *S. aureus*. Agreeing to Kadak and Salem's (2020) research, essential oils and liquor extricates from the seeds of flaxseed, virtuous tree, and dark cumin were ineffectual against.

CONCLUSION

The results appeared that *U. barbata* extricate, a renewable asset recommended as a resistance against the examined bacterial species, may be utilized. Our discoveries infer that encourage examination might grow on the antibacterial examinations and look at how they influence diverse species of microscopic organisms. To get *U. barbata* extricates with important bioactivities for conceivable pharmaceutical employments, encourage considers might improve extraction strategies.

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