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Investigation of Medicinal Activity of Four Imported Trees to Libya Against Some Pathogens

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A B S T R A C T

Many imported trees have been included in Libyan Flora data Base, but not all of its Bio-activity was studied, especially medical in a new environment. Therefore, this work was carried out to Investigate the medicinal activity of four imported trees (*Acaciasaligna*, *Acacia nilotica*, *Brachychiton populneus* and *Leucaena leucocephala*), and evaluate the activity of the aqueous extracts of leaves at a concentration 200mg/ml against four various types of human pathogenic bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Proteus vulgaris*), and Plant-pathogenic fungi (*Aspergillus niger*, *Botrytis cinerea*, *Rhizopus microsporus* and *Fusarium solani*). The antibacterial activity was determined by disk diffusion, and the antifungal activity by poisoned food technique. The results showed the inefficiency of all imported trees leaves extracts against all bacteria types, while, was have good activity against most plant-pathogenic fungi tested, Commonly, *R.microsporus* was the most affected fungus for all the extracts tested, also, the results showed that *L.leuceana* extract is more effective as an antifungal than other extracts. Data in this study indicated the potential of using Imported trees as an environmentally friendly Fungicide.

1 Introduction

Most Arab countries import many ornamental trees, without attention to negative and positive effects (Salih and Abdulrraziq, 2021). Efforts have been made to introduce many of these plants into Libyan flora both deliberately or accidentally, the introduced plants are represented by a total of 361species, 253 genera and 89families, Some are classified as toxic to humans and animals, while others are in fierce competition with native plants, as well as, twenty-nine species were classified as invasive, where these species are becoming established and part of Libyan flora (Alzerbi *et al*., 2020; Mahklouf and Shakman, 2021). Although Several research centers have undertaken studies on the role of native medicinal plants in different fields, little information has been found about medicinal plants. Thus, Should be placed thorough inventory of all potential new medicinal plants in Libya (Agiel and Mericli, 2017; Louhaichi *et al*., 2011). Importantly, if new plant species are included in the import pharmacopeia without replacing native plants, this occupies a hitherto important unconsidered pharmacopoeial niche (Medeiros *et al*., 2012). Fabaceae are one of the biggest families in Libya, represented by 37 medicinal species, 33 species of poisonous, and 9 species ornamental 9 species (Ali *et al*., 2019).

Acacia saligna, *Acacia nilotica*, *Brachychiton populneus* and *Leucaena leucocephala* are among the introduced plants of this family, this species was introduced for a variety of purposes, including food, fodder, erosion control, and afforestation (Alzerbi *et al*., 2020). Apart from that, This family contains chemical constituents of high medicinal value and ethnopharmacologically important, which act in the treatment and/or healing of various body systems (Macedo *et al*., 2018), making an alternative to antibiotics in the treatment of many diseases pathogens

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(Thabet *et al*., 2017; El- Toumy *et al*., 2010; Dzoyem *et al*., 2014). Therefore, This study's objective of verifying the medicinal activity of four trees imported to Libya against some species of bacterial and fungal pathogens, in vitro.

2 Materials and Methods

The study was carried out in the Biology Department/Faculty of education / Omar Al-Mukhtar University. Dried leaves of four imported trees were grinded (*Acacia saligna*, *Acacia nilotica*, *Brachychiton populneus*, *Leucaena leucocephala*) by an electric grinder and saved for use.

2.1 Aqueous Extraction:

200 g of leaves dry powder was added to 1000 ml of sterile distilled water in a glass flask, for each type separately, separately. Hence, Put on a vibratory shaker for 24 hours at 35 ° C, then filter and shake in a centrifuge at 3000 rpm for 10 minutes. The next step was filtering with Whitman No.1 filter paper Filtration was carried out with filter paper on a Buckner funnel by using a vacuum pump. and dried in a Rotary evaporator to get dry powder at a weight of 2.8g (Jigna *et al*., 2005). The concentration of 200 mg/ml was prepared by dissolving 2g of powder in 10 ml of distilled water.

2.2 Test organisms:

Escherichia coli, *Pseudomonas aeruginosa* and *Staphylococcus aureus*: Isolates predefined were obtained from patients reluctant to (Tiba, Alrazi and Altrahum Clinic) laboratories, Al-Bayda / Libya.

Proteus vulgaris: were provided by the bacterial collection, Department of Plant, Omar Al-Mukhtar University.

Fungal isolates (*Aspergillus niger*, *Botrytis cinerea*, *Rhizopus microsporus* and *Fusarium solani*) were provided by the fungal collection, Department of Plant Protection, Omar Al-Mukhtar University.

2.3 Antibacterial activity test:

The mediums were sterilized for 15 minutes in an autoclave at 121°C, bacteria were grown on Mueller-Hinton agar medium. For screening. Sterile filter paper disks (6 mm) impregnated with the extracts were placed on a surface of inoculated bacteria mediums, and used disks impregnated with water as control. The dishes were incubated for 18-24 hours at 37°C with three replications per dish, then a measure of diameters of inhibitory zones minus the diameter of the disc (Driscoll *et al*., 2012).

2.4 Antifungal activity test:

The effect of plant extracts on pathogen growth was determined using poisoned food technique. A volume of 5 ml from each of leaves extracts with a concentration of 200 mg/ml was dispensed separately into 8.5 cm diameter Petri dishes and agitated gently with 45 ml of sterile media PDA. The medium was allowed to be solid and inoculated centrally with 5mm diameter of mycelia plugs of the tested fungi obtained from 7 days old cultures, using a sterile cork borer. Tested fungi were growing on PDA plates inoculated with sterile water that served as control. All cultures were incubated at 28°C and fungal colony diameters were measured daily for 7 days (Singh and Tripathi, 1999). Each experiment was replicated three times. Percentage inhibition as follows:

Percentage inhibition = N1−N2/N1×100

Where, N1 = Radial diameter of fungus in control plates (PDA + Water);

N2 = Radial diameter of fungus in the presence of extracts (PDA + extracts).

3 Statistical Analysis:

The study experiences were designed according to the complete random design (CRD). Statistical analysis was performed using Minitab 17 program and ANOVA variance analysis tables. The averages were compared using Tukey's test at P <0.05 (Abdulrraziq *et al*., 2023).

4 Results

4.1. Effect of imported trees extract against bacteria.

The leaves extracts of four trees imported (*A.saligna*, *A.nilotica*, *B.populneus*, *L.leucocephala*) were tested against different human pathogenic bacteria (Table 1). The results showed no inhibition activity of all the tested tree leaves extracts against *E.coli* and *Ps.aeruginosa*. While *P.vulgaris* recorded weakened sensitivity to extracts *A.nilotica* and *B.populneus* at a diameter (0.7 and 1.0mm), respectively. On the other hand, the most sensitive type to extracts was *S.aureus*, although their sensitivity was weak with diameters ranging from (0.5-1.5mm).

Table (1): Effect of imported trees extract against human pathogens bacteria.

Bacteria / Extract	*E.coli*	*S.aureus*	*Ps.aeruginosa*	*P.vulgaris*
Acacia saligna	-	1.2±0.2 ab	-	-
Acacia noltica	-	0.5±0.0 c	-	0.7±0.1 b
Brachychiton populneus	-	1.0±0.1 b	-	1.0±0.1 a
leauceanaleucocephala	-	1.5±0.0 a	-	-

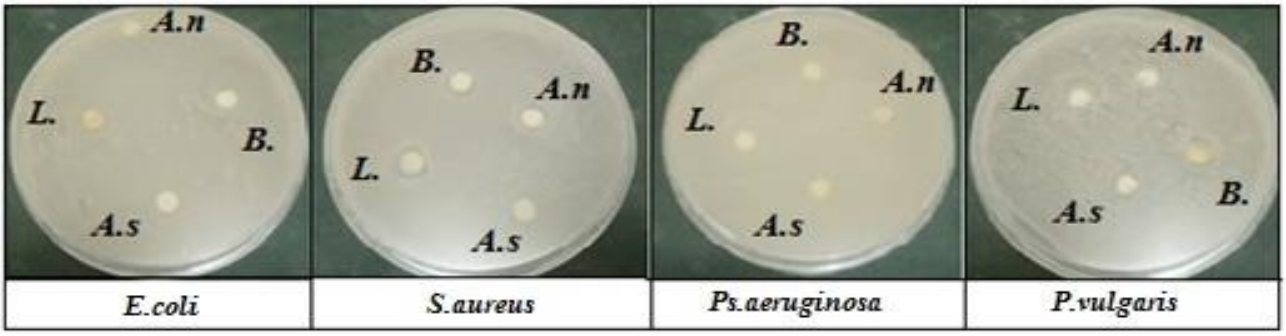

Figure (1): Effect of imported trees extract against types bacteria**.**

***A.n: *A.noltica*; A.s: *A.saligna*; B.: *B.populneus*; L.:*L.leucocephala*.**

4.2. Effect of imported trees extract against fungi.

The leaves extracts of four trees imported (*Acacia saligna*, *Acacia nilotica*, *Brachychiton populneus*, *Leucaena leucocephala*) were tested against different plant pathogenic fungi. The results showed differential effects depending on the extract type and tested fungal species (Table 2). *A.saligna* extract showed no inhibitory effect on the tested fungi except for F.solani (22%). While, *A.nilotica* extract showed inhibition rates (10 and 8%) against *A.niger* and *R.microsporus*, respectively. *B.populneus* extract showed inhibition rates (20, 12 and 16%) against *B.cinerea*, *R.microsporus*, and *F.solani*, respectively. moreover, *L.leucocephala* extract was the best effective against all tested fungi with inhibition rates (60, 55, 68 and 23%) against *A.niger*, *B.cinerea*, *R.microsporus* and *F.solani*, respectively.

Table (1): Effect of imported trees extract against plant pathogens fungi.

Fungi / Extract	*Aspergillus niger*	*Botrytis cinerea*	*Rhizopus microsporus*	*Fusarium solani*
Acacia saligna	-	-	22.0±0.0 b	-
Acacia noltica	10.0±0.0 b	-	8.0±0.0 c	-
Brachychiton populneus	-	20.0±0.0 b	12.0±0.0 c	16.0±0.0 b
leauceanaleucocephala	60.0±0.0 a	55.0±0.0 a	68.0±0.0 a	23.0±0.0 a

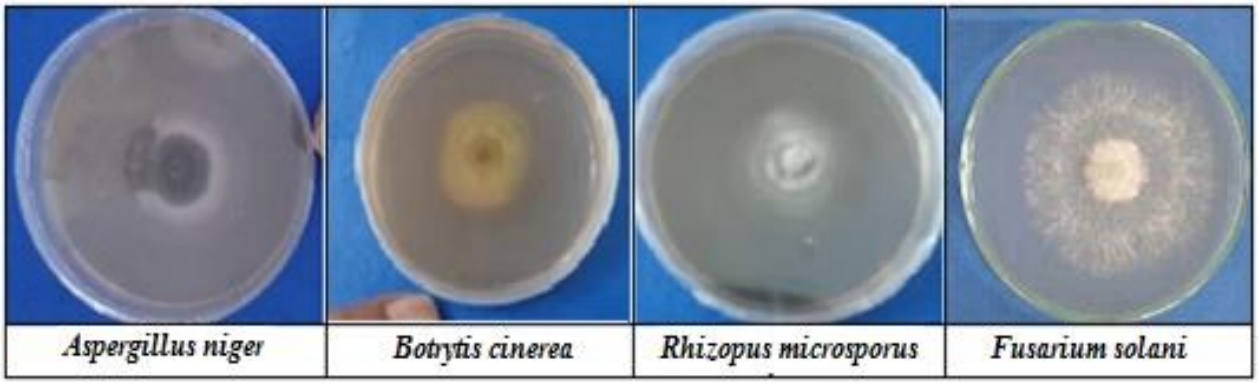

Figure (2): Effect of *Leucaena leucocephala* extract against plant pathogens fungi

5 Discussion

The new species may have the possibility of bioactive compounds, which requires many studies about its bioactivities (Guarim Neto and Morais, 2003). Thus, this study was conducted to commonly show that imported tree extracts possess did not have inhibitory activity against types of bacteria tested. This is evident from the high resistance for *E.coli* and *Ps.aeruginosa* and the weakness of the inhibition diameters, which ranged between (0.5-1.5) for S.aureus and P.vulgaris. disagree this result with (Vijayasanthi *et al*., 2012; Al-Ramamneh *et al*., 2022; Suparno *et al*., 2018), who confirmed that this trees extracts are highly effective in control various types of bacteria. The sensitivity of S. aureus to *L.leucocephala* extract was the highest recorded result against bacterial species at an inhibition diameter (1.5mm), agrees this result with (Saptawati *et al*., 2019), which recommended can be used as an ointment topical anti-*Staphylococcus*. In addition, the results indicate that extracts from these trees have good activity against phytopathogenic fungi, other studies reported good antifungal activity by applying some trees extracts (Banso, 2009; Salem *et al*.,2014). *R.microsporus* was the most affected fungus for all the extracts tested. Finally, leaves extract from *L.leucocephala* showed strong fungicidal activity towards all fungal isolates, especially against *R.microsporus*, *A.niger* and *B.cinerea*, because has several bioactive compounds, which can act as a promising antimicrobial, this result agrees with (Elbanoby *et al*., 2022).

In conclusion: it is easy to import many plants from their original habitat and cultivate them in the local environment for use in different agricultural fields, but the possibility of their exploitation in the medical field may be affected by new environmental factors, and thus become less efficient than it was.

6 Conclusions

This study concluded that the extracts of imported trees (*Acacia saligna*, *Acacia nilotica*, *Brachychiton populneus*, and *Leucaena leucocephala*) had no antibacterial efficiency, on the other hand, they showed good medicinal activity against various types of phytopathogenic fungi, The best inhibition activity of *L.leucocephala* extracts against tested fungi, while, *R.microsporus* was the most affected fungus for all the extracts tested. Findings of this study indicated that the use of imported trees could be a valid alternative for bio-control of plant pathogenic fungi.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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