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Research Article

Expediency of Flea Tree (*Albizia lebbeck L.*) in the prevention of known human fungal and bacterial strains

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Abstract

Albizia lebbeck L. is one of the best medicinal plants in the province of Khyber Pakhtunkhwa Pakistan. It possesses different properties but the most important one, it is used in local remedies. The antibacterial and antifungal properties of different fractions were obtained from the selected plant studied. For Antibacterial activity, four strains of bacteria were selected which cause different diseases in humans, these bacteria were *Streptococcus mutans, Serratia marcescens, Staphylococcus aureus, and Methicillin resistance staphylococcus aureus*. The extract obtained from the *Albizia lebbeck L.*were fractionated into Chloroform, methanolic, *n*-hexane, Ethyl acetate, and Aqueous fractions. The highest antibacterial activity was shown by methanolic extracts (38-48%) and the lowest activity by an aqueous fraction (19-36%). While in antifungal activity the fractions were tested against four fungal species including *Polyspndylium pallidum, Aspergillus flavus, Alternaria alternate,* and *Fusarium oxysporum*. The aqueous extract shows the lowest activity with minimum efficacy in comparison to the standard drug (20-30%), Crude methanolic extract shows a high zone of inhibition followed by *n*-hexane. The study concluded that *A. lebbeck* have significant antimicrobial potential and might be helpful in antibiotics.

Keywords: Antibacterial; Antifungal activity; Antimicrobial; Flea tree; *Albizia lebbeck*

Introduction

It is globally accepted that the first medicine in human history was the medicinal plants **[1]**. Different people of a different time with the help of writing or in other ways disclosed the therapeutic properties of medicinal plants **[2]**. They have many properties in clinics from cough to cancer. From the 18th century, the pharmacologist was busy to find medicinal plants and knowledge about their constituents to use against different diseases. They consist of two types of metabolites i.e. primary and secondary metabolites **[3, 4]**. The primary metabolites help in growth and

development while secondary metabolites run many biochemical pathways and give therapeutic property to plants [5]. The fractions obtained from the selected plant show the therapeutic properties against different pathogens including bacteria and fungi [6]. The medicinal plants were used in different parts of the world but in Asia, it is used about 80%. It is also used in Latin America and Africa with minimal side effects [7]. According to some research such as the World health organization (WHO), in the whole world population, about 80% used traditional medicine which is prepared from medicinal plants [8]. For millennia it is known that medicinal plants have the potential to fight against microorganisms and people of different countries especially China, India, and the northeast were initially known to prepare drugs from the medicinal plants [9]. But with the passage of time and the failure of therapies these drugs were failed against microbes. After that different precuts were used against them which shows a very significant role against them. Fungi and Bacteria have a dual threatening effect because they not only diseased our economic crops but also causing toxicities and allergies. Fungicides that fight against fungal diseases prepared from medicinal plants [10]. In this regard, the present study was held to find anti-fungal and anti-bacterial activity.

Albizia lebbeck L. is a dioecious plant with compound leaves fruit is flat and oblong and distributed in Australia, Asia, Africa and South America [11]. The seed is round and colorful and usually grown as a wild plant. It also possesses therapeutic properties. The fractions obtained from the plant showed antifungal and antibacterial activity. These different extracts such as methanolic and Ethanolic fractions possess different properties against certain diseases such as anticancer, hypoglycemic, antiprotozoal, and anti-fertility effect [9, 12]. While on the other hand traditionally it is used against pain, inflammatory, analgesic [13]. The drugs which are prepared from the plants have the potential to use against certain diseases of human beings which are caused by microorganism especially bacteria and fungi [14].

Materials and Methods

Collection and Processing of the Plant Specimen

A. lebbeck L. collected from different areas of Peshawar, KP, Pakistan. After collection, the plants were washed with water, dried, in newspapers to absorb moisture from the plants. For further drying, they were placed in the oven for 24hrs under 40^0C. When the plants were completely dried they were ground by grinder for powder formation. The powder was placed in a dried and cool area for further activities.

Preparation of Extract and Fractionation

Active metabolites were extracted using the cold maceration method. In two liters' ethanol one and a half kilogram of powdered which was made from the selected plant was dipped and incubated at 25°C. After that, the filtrate obtained by filtration of mixture thrice using filter paper. For evaporation then it was subjected to a rotary evaporator at 40°C. Then it is again dried and dissolved in 100ml of distilled water. For fractionation different organic solvents such as ethanol, methanol, and n-hexane were used. All the fractions thus obtained were concentrated by rotary evaporator and designated as solvent-extracted fractions [15].

Test Microorganism

A total of four bacterial and four fungal species were tested. The bacteria species *Streptococcus mutans, Serratia marcescens, Staphylococcus aureus,* and *Methicillin resistance staphylococcus aureus (*MRSA) which cause diseases in human beings and the fungal species are *Polyspndylium pallidum, Fusarium oxysporum, Alternaria alternate and Aspergillus flavus* [15].

Antibacterial bioassay

Antibacterial activity of crude extracts was carried out in Petri dishes by disc diffusion method [12]. 6mg/ml of dilute culture was transferred to the petri dish and placed at 37°C for 30 min. After transferring 8mm diameter disc was used for soaking of differently concentrated solutions. For positive control, Gentamicin, 20 µg, and Gatifloxacin 20 µg was used while for negative control soaked disc with distilled water is used. The disc was placed in plates and incubated for 24 hr at 37°C. The inhibition zone was measured after the completion of incubation [15].

Antifungal bioassay

During antifungal activity four fungal strains *Polyspndylium pallidum, Fusarium oxysporum, Alternaria. alternate and Aspergillus flavus* were studied. Sabouraud agar medium was used in Petri dishes for such activity. 6mg/ml fractions were poured into each plate and the same size disc which was used for antibacterial activity was used. For incubation, such plates were placed for one day at human body temperature (37°C). When the incubation period was completed inhibition zone was measured [16].

Statistical analysis

The statistical analysis was done through ANOVA and SPSS data analysis.

Results

The focused antimicrobial activities of different fractions of *A. lebbeck* L. revealed momentous effects against different bacterial and fungal strains of humans. The biochemical must be followed to isolate substantial compounds for the preparation of antibiotics [17]. For this purpose, antimicrobial activities were carried out.

Antibacterial activity

In our results the antibacterial properties (Fig. 1 & 2) of *A. lebbeck.* Crude Methanolic extract inhibited the growth of *S. aureus* (10.3 ± 0.65mm with 38.47%), *S. mutans* (12.4 ±0.56 mm and 43%), MRSA was restricted (12.1 ±0.89 mm and 48.5%), and *S. marcescens* (09±0.68 mm and 42.87%). The restriction showed by *n*-hexane fraction was *S. aureus* (09.3±0.36mm and 31%), *S. mutans* (11±0.79 mm and 36%), *MRSA* (09±0.64 mm and 32.02%), and *S. marcescens* (11 ±0.93 mm and 47.63%). The Chloroform fraction limited bacterial growth 9.4 ±0.43 mm zone and 34.72%, 11.0±0.56 mm and 44%, 07 ±0.44 mm and 25% and 09±0.55 mm zone and 42.86% of *Staphylococcus aureus, Streptococcus mutans, Methicillin resistance staphylococcus aureus* (MRSA) and *Serratia marcescens.* Ethyl acetate fraction restricted *Staphylococcus aureus* to (12 ±0. 27 mm and 46.16%), the *MRSA* strain to (07±0.19 mm and 28.03%). While *S. mutans* was restricted to (12.5 ±0.11mm and 43%), and *S. marcescens* (11±0.16 mm and 48.33%). Outer limits of zone inhibition of Aqueous fraction were *S. aureus* (05.5±0.47 mm and 19.53%), MRSA (9.4 ±0.56 mm and 35.90%), *S. mutans* (7.90 ±0.69 mm zone and 29.11%), and *S. marcescens* (7.4 ±0.78 mm, and 29.38%). The statistical analysis of data shows that one factor ANOVA of antibacterial contains the value of P= 1.94x 10^{-09} which is very much significant.

Antifungal Activity of *Albizia lebbeck* L.

The statistical analysis of data shows that one factor ANOVA of antifungal contains the value of P= 2.71x 10^{-10} which is very much significant. The trend of results showed that efficacy against selected was more dependent on the kinds of fraction rather than the type of pathogen. In our results, the antifungal properties (Fig. 3) of *A. lebbeck L.*Crude Methanolic extract inhibited the growth of *Alternaria alternate* by 56±0.58 mm as highest and restricted *Polyspndylium pallidum* by 40.0±0.46 mm as lowest. The restriction showed by *n*-hexane fraction were, *Fusarium oxysporum* (52±0.48 mm), *Alternaria alternate* (50±0.54), *Polysphndylium pallidum* (47.0±0.63 mm), and *Aspergillus falvus* (35±0.69 mm)

correspondingly. The Chloroform fraction limited fungal growth to 60±0.34 mm, 55±0.65 mm, 50±0.37 mm, and 45±0.31mm of *Alternaria alternate*, *Fusarium oxysporum*, *Polyspndylium pallidum*, and *Aspergillus flavus* correspondingly. Ethyl acetate fraction restricted *Alternaria alternate*, (55±0.47 mm), *Fusarium oxysporum* to (45±0.43 mm), *Aspergillus flavus* to (40±0.71), and *Polysphndylium pallidum* (30±0.57 mm). Outer limits of zone inhibition of aqueous fraction were *Alternaria alternate* to (20.0±0.52 mm), followed by *Polyspndylium pallidum*, (25.0 ±0.64 mm) *Fusarium oxysporum* (25.0±0.53 mm), and *Aspergillus flavus* to (30.0±0.56 mm).

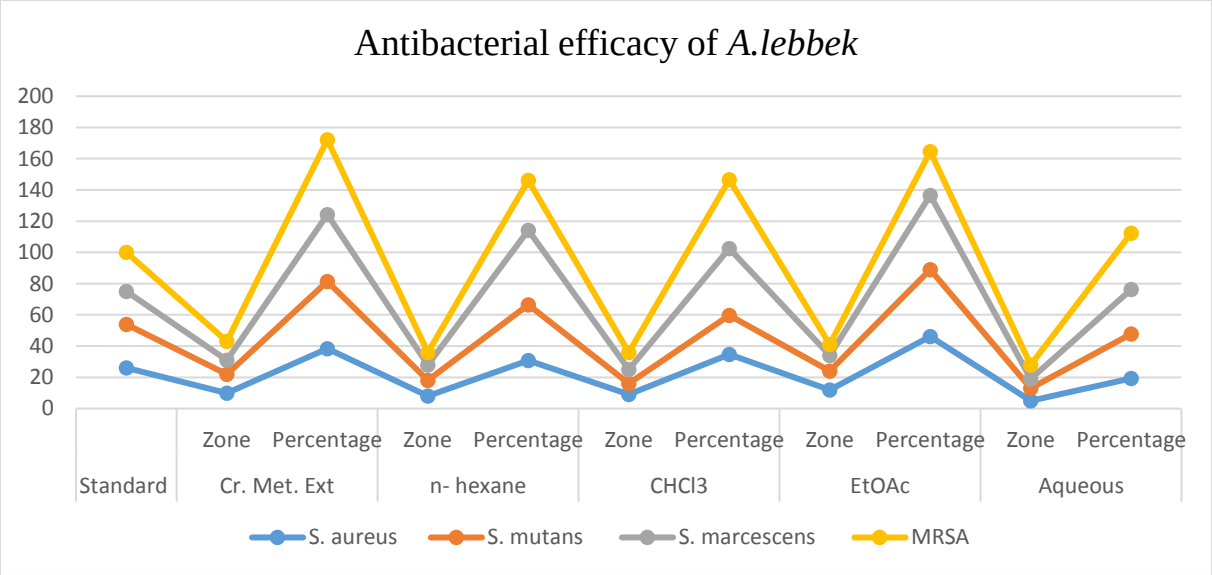

Figure 1. Antibacterial Efficacy and Percentages of Zone of Inhibitions

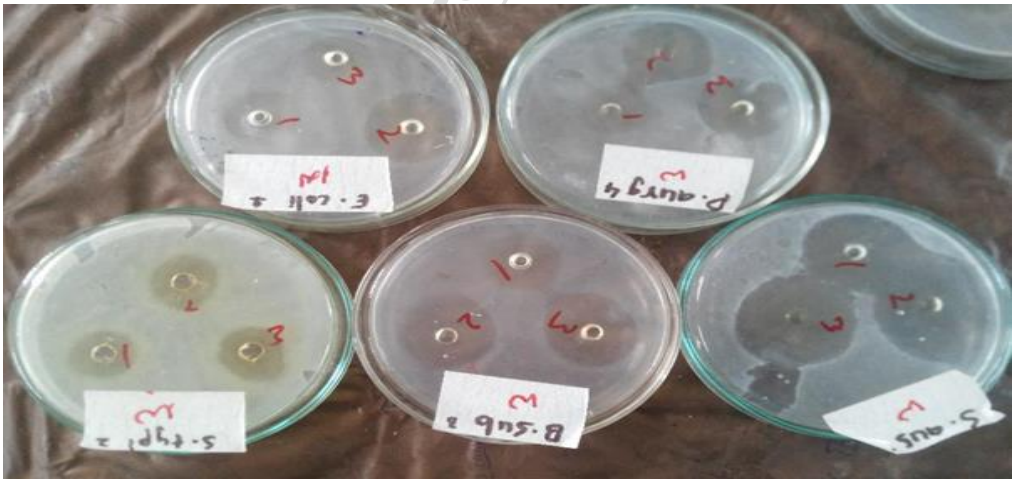

Figure 2. Antibacterial activity of *Albizia lebbeck* L.

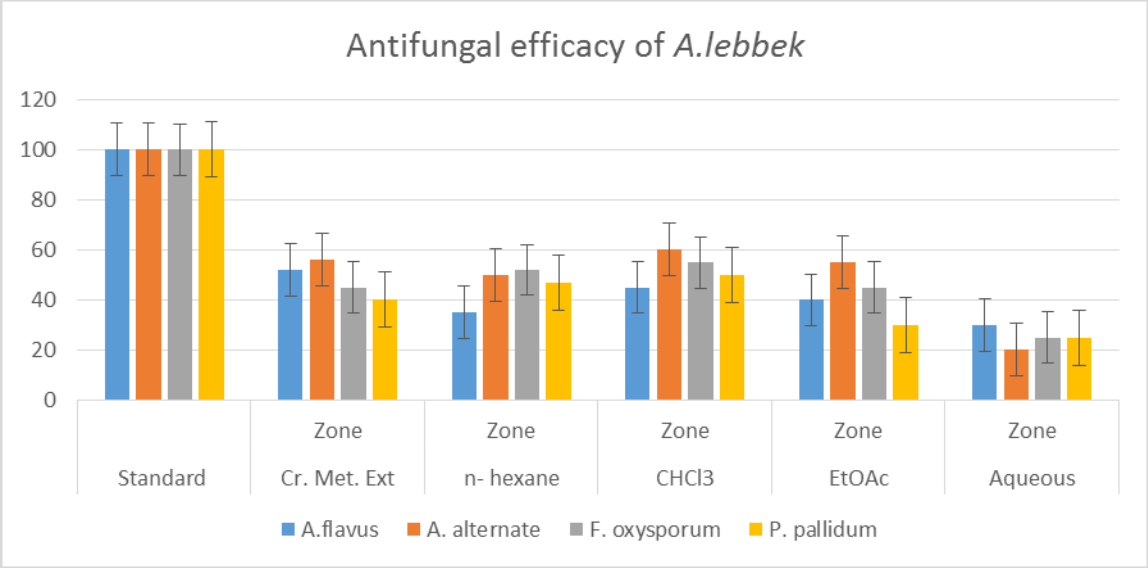

Figure 3. Antifungal Efficacy of Flea tree (*Albizia lebbeck* L.)

Discussion

Antibacterial activity

The selected bacterial pathogens were *Serratia marcescens, Staphylococcus aureus, Methicillin resistance staphylococcus aureus* (MRSA), *and Streptococcus mutans.* A 10 mg/ml of the selected medicinal plant fractions were tried out on selected pathogens. The results indicated that all fractions of the selected plant were significantly effective against the selected human pathogens. The statistical analysis of data shows that one factor ANOVA of antibacterial contains the value of P= 1.94x 10^{-09} (Table 1) which is very much significant. The trend of results showed that efficacy against selected was more dependent on the kinds of fraction rather than the type of pathogen.

Antifungal activity

The fractionated extracts were tested on common human bacterial and fungal pathogens, which were collected from hospitals. The selected bacterial pathogens were *Polyspndylium pallidum, Fusarium oxysporum, Alternaria. alternate and Aspergillus flavus* . A 10 mg/ml of the selected medicinal plant fractions were tried out on selected pathogens. The results indicated that all fractions of the selected plant were significantly effective against the selected human pathogens which are following findings of **[18]**. The statistical analysis of data shows that one factor ANOVA of antibacterial contains the value of P= 1.94x 10^{-09} which is very much significant. The trend of results showed that efficacy against selected was more dependent on the kinds of fraction rather than the type of pathogen **[19, 20]**. The statistical analysis of data shows that one factor ANOVA of antifungal contains the value of P= 2.71x 10^{-10} (Table 2) which is very much significant **[21-23]**. The trend of results showed that efficacy against selected was more dependent on the kinds of fraction rather than the type of pathogen **[24, 25]**. In conclusion, it is very much clear now the plant has a strong antimicrobial potential **[26]** and has very significant results against bacteria and fungi.

Table 1. Single-factor ANOVA of Antibacterial efficacy of Flea tree (*Albizia lebbeck* L.)

Summary						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Standard	4	100	25	8.666667		
Cr. Met. Ext	4	43	10.75	2.25		
n- hexane	4	36	9	1.333333		
CHCl3	4	36	9	2.666667		
EtOAc	4	41	10.25	5.583333		
Aqueous	4	28	7	3.333333		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	865.8333	5	173.1667	43.59441	1.94E-09	2.772853
Within Groups	71.5	18	3.972222			
Total	937.3333	23				

Table 2. Single-factor ANOVA of Antifungal efficacy of Flea tree (*Albizia lebbeck* L.)

Summary						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Standard	4	400	100	0		
Cr. Met. Ext	4	193	48.25	50.91667		
n- hexane	4	184	46	58		
CHCl3	4	210	52.5	41.66667		
EtOAc	4	170	42.5	108.3333		
Aqueous	4	100	25	16.66667		
ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	12690.88	5	2538.175	55.26114	2.71E-10	2.772853
Within Groups	826.75	18	45.93055556			
Total	13517.63	23				

Conclusion

The present study focused on the antibacterial and antifungal activities that showed significant results through different fractions against different bacterial and fungal strains and might be recommended for molecular and compound level studies.

Author's contribution

Conceived and designed the experiments: M Hamayun, Performed the experiments: M Hamayun, T Yaseen & KU Rehman, Analyzed the data: KU Rehman & S Ullah, Contributed materials/ analysis/ tools: ZA Butt, S Khan & T Khan, Wrote the paper: KU Rehman, S Ullah & G Ahmad.

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