In vitro Evaluation of Anti-Microbial Potential of the Leaf Extracts of *Acacia modesta*

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Summary: Infectious diseases caused by micro-organisms are transmissible and infect a large group of population. Investigations were carried out for studying the phytochemistry and biological potential of the leaves of *Acacia modesta*. Dried and finely ground leaves were extracted with ethanol. Different fractions were obtained by extracting the crude extract with n-hexane, chloroform and ethyl acetate. Fractions of n-hexane, chloroform, ethyl acetate, crude extract and the aqueous layer left behind were evaluated for their anti-microbial potential by determining the zone of inhibition against different bacterial strains. All fractions showed positive anti-bacterial activity except ethyl acetate fraction. However the aqueous layer showed activity which is significantly higher than the standard antibiotics used in this study. In conclusion the more active the compounds found in leaves, the more polar they were in nature. Bioassay guided isolation of these active compounds from aqueous fraction may lead to potential anti-bacterial metabolites from *Acacia modesta*.

Keywords: *Acacia modesta*; Phytochemical screening; *In vitro* bioassay, Biological screening, Anti-microbial activity; Anti-bacterial activity.

Introduction

Natural products are the final products of nature's laboratories where millions of compounds are being synthesized having interesting biological activities [1]. A world of literature is available on the discovery of bioactive compounds from natural sources, especially plants [2,3], which have been explored for obtaining compounds having an array of different biological activities such as anti-diabetic [4-6], anti-cancer [7-9], anti-oxidant [4,10-12], anti-inflammatory [13-17], enzyme inhibitory [18-21], anti-microbial [22-25] etc.

Acacia modesta belongs to the genus *Acacia* having about 163 species. The pharmacological importance of genus has been explored by bioactivity guided isolation, purification and identification of active compounds using different solvent extracts [26-34]. Bioassay screening of the extracts of different parts of *Acacia modesta* has been carried out [35-38], depicting its pharmacological importance.

The present study is mainly concerned with the biological studies of various fractions obtained from *Acacia modesta* leaves, aiming to screen some microbial species that have not been previously screened for this plant and subsequently develop a comprehensive picture for the bioassay guided isolation of biologically active metabolites.

Results and Discussion

Extraction and Fractionation

The dried leaves of *Acacia modesta* were ground and extracted with ethanol. The crude ethanolic extract was fractionated using different solvents by liquid-liquid extraction technique.

All the fractions were subjected to bioassay screening.

Bioassay Screening

Anti-microbial potential of the crude extract and fractions was evaluated by agar well diffusion assay against selected microbes. Among all extracts, the aqueous fraction which was obtained after the separation of all other fractions was found to be most active against *P. aeruginosa* (20 ± SD mm), *A. baumanni* (14.5 ± SD mm), *and M. morganii* (16 ± SD mm). The n-hexane fraction showed activity against *E. coli* (12 ± SD mm), *A. baumanni* (13 ± SD mm), *P. aeruginosa* (12 ± SD mm), *and H. influenzae* (13 ± SD mm). The ethyl acetate fraction was found inactive against all the strains used in the current study. The growth of *P. aeruginosa* (12 ± SD mm), *A. baumanni* (12 ± SD mm), *M. morganii* (12 ± SD mm) and *H. influenzae* (12 ± SD mm) were inhibited moderately by the chloroform fraction. However the crude extract was active against *P. aeruginosa* (15.5 ± SD mm). (Table-1).

Table-1: Zone of Inhibition (mm) of different fractions obtained from the crude extract of *A. modesta* against bacterial strains.

Microbes / Samples	*E. coli*	*M. morganii*	*P. aeruginosa*	*K. pneumonia*	*A. baumanni*	*H. influenzae*	*S. aureus*
1*	-	-	15.5	-	-	-	-
2**	12	-	12	-	13	13	-
3***	-	12	12	-	12	12	-
4****	-	-	-	-	-	-	-
5*****	-	16	20	-	14.5	-	-
STREPTOMYCIN	15	-	-	16	15	17	20
AMPICILLIN	18	-	18	-	20	20	-

*ethanolic extract
**n-hexane fraction
***chloroform fraction
****ethyl acetate fraction
*****Aqueous layer.

The graphical representation (Fig. 1) of the study shows that the aqueous layer was more active than any other fraction. None of the fractions showed any activity against *K. pneumonia* and *S. aureus*. *M. morganii* was resistant to both ampicillin and streptomycin but its growth was inhibited by the chloroform fraction (12 ± SD mm) and aqueous layer (16 ± SD mm), which is an interesting finding. *P. aeruginosa* is resistant to ampicillin and was inhibited by streptomycin (18 ± SD mm), while the aqueous layer showed greater zone of inhibition (20 ± SD mm) than both the standards against this strain.

Experimental

General

Commercial grade solvents were used after distillation. Thin layer chromatography (TLC) was carried out using pre-coated silica gel plates (G-25-UV254) and visualized under ultra violet (UV) light at 254 nm. Extracts were concentrated on Buchi R-210, rotary evaporator.

Extraction and Fractionation

Acacia modesta plant was collected from the suburbs of district Mardan, Khyber Pakhtunkhwa, Pakistan. Twigs were removed from the leaves and were left under shade to dry. The dried leaves were ground to fine powder (~ 10 Kg) and soaked in ethanol for 3 days. Ethanolic extract was removed and concentrated under reduced pressure on a rotary evaporator.

The crude ethanolic extract (750 g) was dissolved in water and fractionated thrice with different solvents to get n-hexane, chloroform, and ethyl acetate fractions. The extracts were subjected to evaluation of their anti-microbial potential.

Biological Activities

The crude extract and the fractions were evaluated for their anti-microbial potential.

Microbial Cultures

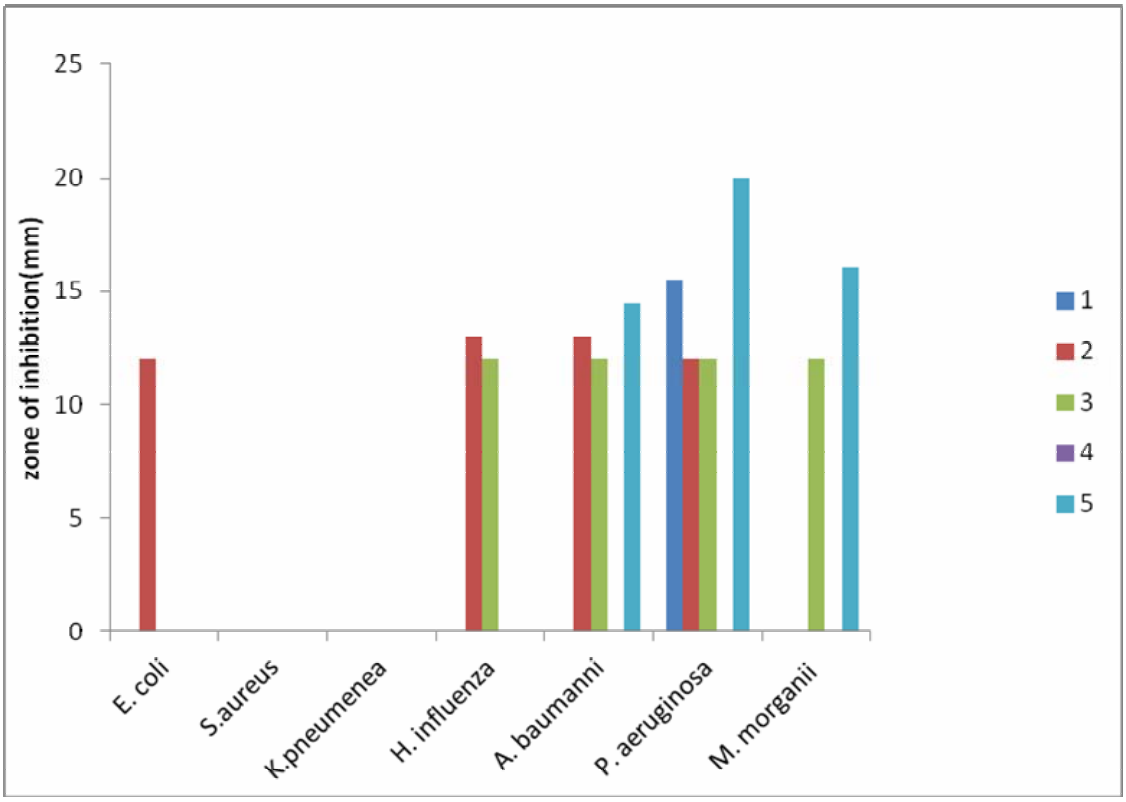
Anti-microbial activity was assessed against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsella pneumonia*, *Acinetobacterus baumanni*, *Marginella morganii* and *Haemophilus influenzae*. Microbial strains were obtained from the National Institute of Health (NIH), Islamabad, Pakistan.

Preparation of Culture Media

The culture media was prepared by dissolving 20 g of LB-broth media (Sigma-Aldrich, USA) in 1000 mL of distilled water. The turbid solution obtained was heated to obtain a clear and transparent solution with continuous shaking. The media was sterilized in autoclave at 121 °C for 15 minutes at 15 lbs pressure. Sterilized media was poured in petri dishes (30 mL each) under aseptic conditions and let it to solidify at room temperature.

Culture Preparation

All the microorganisms were sub-cultured using streaking method. Inoculums of all microbes were prepared in sterilized LB-broth media. 20 g of LB-broth powder was taken in 1 L of distilled water and autoclaved for 15 minutes at 121 ^{o}C. The sterilized liquid media (5 mL) was then poured in separate test tubes and let to cool at room temperature. The bacterial inoculums obtained were transferred to the media filled test tube and placed in shaker incubator at 37 ºC for 24 hours. Optical density of each culture was recorded at 660 nm. Absorbance of 0.5-1.0 was considered as optimal growth for anti-microbial activity determination.



1) ethanolic extract **2)** n- hexane fraction **3)** chloroform fraction **4)** ethyl acetate fraction **5)** Aqueous layer.

Fig. 1: Chart showing the results of anti-microbial activity.

Anti-Microbial Assay

Anti-microbial activity was evaluated by agar well diffusion method. A 75 µL of each microbial culture was spread individually on each plate. Wells of 9 mm were bored in each plate. 100 µL of each sample was distributed in individual well. Negative control (DMSO) and a positive control (antibiotic well) were used, assigned as a standard. The plates were incubated at 37 ºC for 24 hours. After 24 hours, zones of inhibition were measured and expressed in millimeter.

Conclusion

The result of anti-bacterial activity shows that the crude extract was only active against *P. aeruginosa*, while its fractions showed activity against other strains of bacteria as well. This loss of activity in the crude extract might be due to the antagonistic effect of so many compounds present. The aqueous layer left after the separation of the entire fractions significantly inhibited the growth of *M. morganii*, *A. baumanni* and *P. aeruginosa* as compared to the standards streptomycin and ampicillin used in current study. The aqueous layer needs to be studied more closely for the isolation of these anti-bacterial constituents.

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