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EVALUATION OF ANTIBACTERIAL ACTIVITY OF *ACACIA MODESTA*

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ABSTRACT

Objective: The objective of the study was to evaluate in-*vitro* efficacy of antibacterial activity of crude methanol, n-hexane, chloroform, ethyl acetate and aqueous extracts of *Acacia modesta*againstsome human pathogenic bacterial strains.*Acacia modesta*, commonly known as *Phulai*, is a member of the family **Leguminosae)** and sub-family **Mimosaceae.** It is a deciduous, slow-growing small tree with thorny young shoots and dark brown and black wood. The plant is also popular in herbal medicines, including those for the treatment of muscular conditions, back pain, and stomach problems. **Methodology:** Crude methanol, n-hexane, chloroform, ethyl acetate and aqueous extracts of *Acacia modesta*were used for antibacterial screening. Antibacterial activity was tested against pathogenic bacterial strains of *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Bacillus Pumilus, Klebsiellapneumoniae, Enterobacteraerogenes*and *Streptococcus pneumonia.*Antibacterial activity of Acacia *modesta* extract was carried out by using disc diffusion method. **Results**: The crude methanolic extract showed moderate activity against *E. coli* (40.74%), *P. aeruginosa* (40.74%) and *B. pumalis* (40%), low against *S. epidermidis* (34.61%), *S. typhi* (22.22%), *S. pneumoniae*(27.58%) and *E. aerogens* (31.03%) and no activity against *S. aureus* and *K. pneumoniae*. The n-hexane fraction was significantly active against *K. pneumoniae* (66.66%) and moderately active against *E. coli* (48.14%), *S. typhi* (51.85%), *P. aeruginosa* (51.85%) and *B. pumalis* (40%). It showed low activity against *S. epidermidis* (34.61%), *S. pneumoniae* (20.68%), *S. aureus* (38.46%) and *E. aerogens* (34.48%). Chloroform fraction was moderately active against *K. pneumoniae* (57.14%), *S. typhi* (48.14%), *E .aerogens* (41.37%) and *B. pumalis (40%)* and low active against *S. aureus (38.46%), P. aerugenosa (37.03%), S. epidermidis (34.61%), E. coli (33.33%)* and *S. pneumoniae (24.13%).* Significant activity was shown by the*EtOAc*fraction against *K. pneumoniae (61.90%),* moderate against *S. typhi (48.14%), E. coli (44.44%), P. aeruginosa (44.44%)* and *B. pumalis (40%)* while low activity against *E. aerogens (37.93%), S. aureus (34.61%), S. epidermidis (34.61%)* and *S. pneumoniae (24.13%).* The aqueous fraction showed moderate activity against *S. epidermidis (53.84%), B. pumalis (44%), E. coli (48.14%)* and *S. typhi (40.74%),* low activity against *K. pneumoniae (38.09%), P. aeruginosa (37.03%), E. aerogens (37.93%), S. aureus (34.61%)* and no activity against *S. pneumoniae.*The n-hexane and EtOAc fractions exhibited significant while $CHCl_3$fraction showed moderate activity against *K. pneumoniae*. The aqueous fraction showed low activity against the majority of test pathogens.The crude methanolic extract have low activity against most of the test pathogens. **Conclusions:** The results concludes that n-hexane, ethyl acetate extracts of *Acacia modesta* possess antibacterial activity.

KEYWORDS: *Acacia modesta*, Antibacterial activity, *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Bacillus Pumalis, Klebsiellapneumoniae, Enterobacteraerogene.*

INTRODUCTION

About 60% of the world's populations exclusively rely on traditional medicine (plant extracts) for their primary health-care needs (Farnsworth, 1994). Currently, there are increasing incidents of infections due to evolution of new pathogens and resistance of the present pathogens to the existing antibiotics, for example, multi-drug resistant tuberculosis (MDR-TB) is resistant at least to isoniazid and rifampicin, the two most powerful first-line anti-TB drugs (Leimane., 2005). Plants are rich sources of bioactive compounds. Of the world's 25 bestselling pharmaceutical agents, 12 are naturally derived products. In view of this, an attempt has been made to study the antibacterial activity of herbal drug.

Acacia modesta belongs to family Fabaceae (subfamily Mimosaceae). It is commonly known as phulai and locally called palosa. It is distributed in India and Pakistan. The wood of *A. modesta* is durable and hard. It is used to treat leprosy, wounds, dysentery and venereal diseases.[1-4] traditionally, ash of *A. modesta* wood was employed for relieving severe body pain. Mixture of gum with wheat flour, almond and butter was given to women after delivery. Zhublesharbat, solution of gum in water, was taken as health stimulant. Due to antimicrobial properties of *A. modesta*, its branches were used as tooth brush. Because of curative properties, gum was used for back pain and sex. Plant use was also seen in treatment of cough.[5-9]

*A.modesta*is a deciduous tree which has medium or small size. Bark is rough and greenish grey. Prickles are below petioles and in the form of pairs. These prickles are dark brown, compressed, shinning, recurved and 4 to 5 mm in length while they may be absent in some cases. Pinnae are usually in pairs (2-3) and hardly 1. Leaflets are also in pairs (3-5), petiolulate, obovate, obtuse, oblique, glaucous and noticeable veins. Inflorescence is pedunculate spike (3.7-7.5 cm length) while peduncle is 1.3-2.5cm. Calyx is glabrous and broadly campanulate, with 1-1.5 mm length. Corolla's length is 2-2.5 mm. Stamens are many. Pods are flat, straight, mucronate, glabrous, stipe and stipitate with variable length ranging from 5-10 mm. Number of seeds varies from 3-5 and flowering season is March to May.[10-11]

MATERIALS AND METHODS

Collection and authentication

Acacia modesta wascollected from in and around Chembarambakkam, Chennai, India. The plant was identified and authenticated by the taxonomist. The authenticated specimen was deposited in the Department of Pharmacognosy, SreeSastha Pharmacy College. The authentication specimen number is SSPC/P.COG/001/2020. The aerial parts were dried in room temperature for 2 months. Dried specimen was powdered using mechanical grinder and passed through 60 mesh sieve to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Preparation of extract

The powdered material of *A.modesta*7 kg) was soaked in methanol for 15 days, twice, at room temperature, with occasional shaking. Each time, the material was filtered and the filtrate was concentrated at 40°C under vacuum, by rotary evaporator. A blackish crude methanolic extract of *A.modesta* (850 g) was obtained.

Fractionation

The crude methanol extract of *A.modesta* (1000 g) was suspended in distilled water (5000 ml) and partitioned with n-hexane (3 x 500 ml), chloroform (3 x 500 ml) and ethyl acetate (3 x 500 ml), respectively, to yield the n-hexane (200 g), chloroform (160 g), ethyl acetate (110 g) and aqueous (240 g) fractions. 90 g of the crude methanolic extract of *A.modesta* was left for biological/pharmacological activities. All the fractions will only contain their particular compounds based on the solubility from the crude extract. For example, the n-hexane fraction will contain only those compounds which are non-polar, and so an.

Antibacterial activity

Determining percent inhibition[12-20]

Antibacterial activity of the crude methanol,n-hexane,chloroform,ethylacetate and aqueous extracts of *A.modesta*were determined against *Escherichia coli, Pseudomonas aeruginosa, S. aureus, Staphylococcus epidermidis, Salmonella typhi, Bacillus pumalis, Klebsella pneumoniae, Streptococcus pneumoniae and Enterobacter aerogenes* (Ahmad et al., 2009). Eighteen hours old culture of the test organism from the nutrient broth was transferred to sterile nutrient agar plates to make bacterial lawn. After 30 min, using a sterile 6 mm borer, wells were dug in plates. Stock solutions (3 mg/ml) of the test samples were prepared in sterile dimethyl sulfoxide (DMSO, less than 1%). 100 µl of crude methanolic extract and fractions were loaded to their respective wells. Amoxicillin and DMSO (less than 1%) were used as positive and negative controls, respectively. Zone of inhibition was measured (in mm) in comparison with positive control using the following formula.

$$\% \text{ Inhibition} = \frac{\text{Zone of Inhibition of Sample} \times 100}{\text{Zone of Inhibition of Standard}}$$

Determination of minimum inhibitory concentration (MIC)

After determining the percent inhibition, the MIC50 of the test samples at the concentration of 0.9, 1.5, 2.1, 2.7 and 3.2 mg/ml were measured against the test organisms (Banso, 2009). To sterile nutrient broth in the test tubes (4 ml), test samples and test organisms were inoculated, incubated for 24 h at 37°C. Results were recorded after 24 h based on the percent clarity against negative control. Negative control in this case was nutrient broth media.

RESULTS AND DISCUSSION

Table 1: Antibacterial activity of crude methanolic extract & various fractions of *Acacia modesta.*

Name of Bacteria	Zone of Inhibition of standard(Amoxicillin) 10µg/Disc	Crude Extract Methanol		*n*-hexane		$CHCl_3$		EtOAc		Aqueous	
		Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)	Zone of Inhibition (mm)	Inhibition (%)
S.epidermidis	26	9	34.61	9	34.61	9	34.61	9	34.61	14	53.84
S.typhi	27	6	22.22	14	51.85	13	48.14	13	48.14	11	40.74
S.pneumoniae	29	8	27.58	6	20.68	7	24.13	7	24.13	0	0
S.aureus	26	0	0	10	38.46	10	38.46	9	34.61	9	34.61
P.aeruginosa	27	11	40.74	14	51.85	10	37.03	12	44.44	10	37.03
K.pneumoniae	21	0	0	14	66.66	12	57.14	13	61.90	8	38.09
B.pumalis	25	10	40	10	40	10	40	10	40	11	44
E.aerogens	29	9	31.03	10	34.48	12	41.37	11	37.93	11	37.93
E.coli	27	11	40.74	13	48.14	9	33.33	12	44.44	13	48.14

Table 2: MIC_{50} values (mg/ml)of crude methanolic extract and various fractions of *Acacia modesta.*

Name of Bacteria	Crude extract	n-hexane	$CHCl_3$	EtOAc	Aqueous
S.epidermidis	3.5	3.2	3.5	3.4	2.9
S.typhi	3.7	2.7	2.9	2.7	3.2
S.pneumonia	3.7	3.9	3.9	3.5	0
S.aureus	0	3.2	3.2	3.5	3.8
P.aeruginosa	3.2	2.7	3.2	3	3.7
K.pneumoniae	0	2.4	2.5	2.4	3.5
B.pumalis	3.2	3.2	3.2	3.2	2.9
E.aerogens	3.9	3.6	3.2	3.9	3.9
E.coli	3.2	2.7	3.5	2.9	2.7

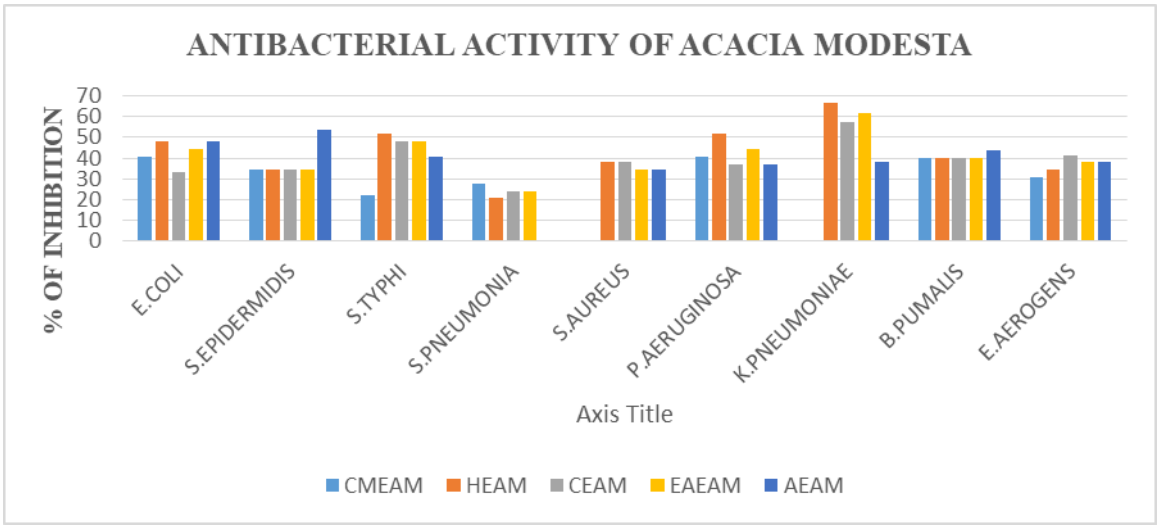

Fig. 1: Antibacterial activity of crude methanolic extract & various fractions of *Acacia modesta.*

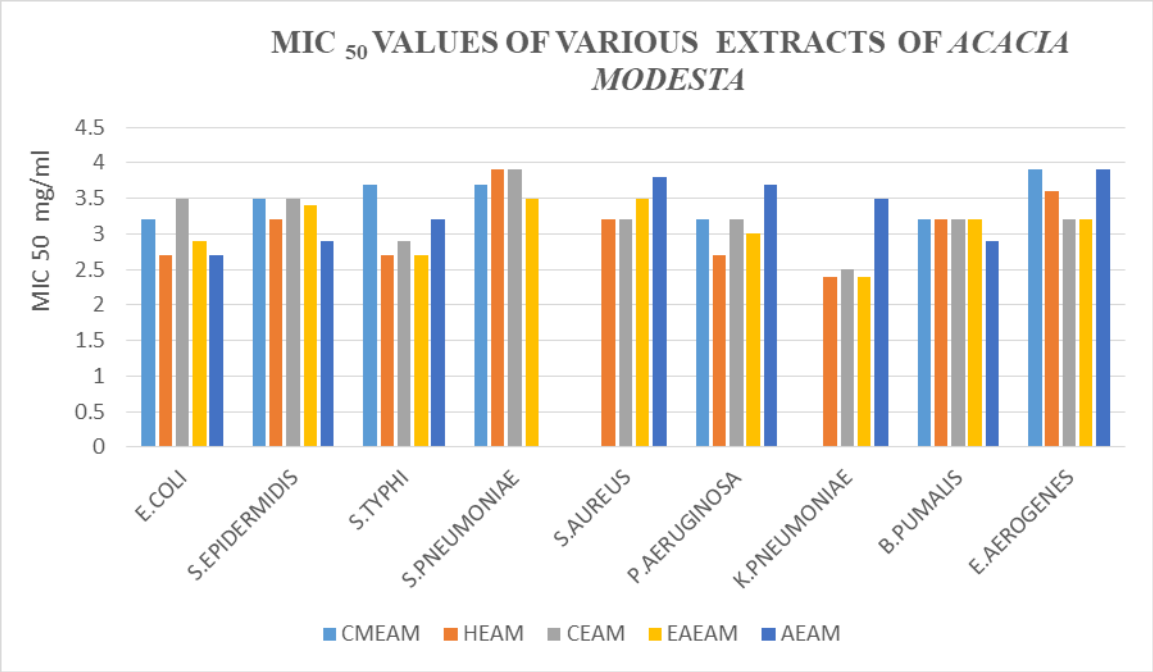

Fig. 2: MIC_{50} values (mg/ml) of crude methanolic extract and various fractions of *Acacia modesta.*

The interest regarding the research on medicinal plants has increased over the last few decades due to onset of new infection, in particular, infections by *Enterococcus* and *Staphylococcus* species, which are agents of many intra-hospital infections and antibiotic resistance to available drugs, e.g. *S. aureus* has become resistant to several antibiotics to which it was previously susceptible. Some of the antibiotics to which it is now resistant are pencillin G, macrolides, lincosamides, tetracyclines and gentamicin (Ayliffe, 1997). With the intent of exploring new bioactive compounds from plant origin, The results of antibacterial activity of the crude methanol, n-hexane, chloroform, ethylacetate and aqueous extracts of *A.modesta* are shown in Table-1 and Fig 1.The crude methanolic extract have low activity against most of the test pathogens. The crude methanolic extract showed moderate activity against *E. coli* (40.74%), *P. aeruginosa* (40.74%) and *B. pumalis* (40%), low against *S. epidermidis* (34.61%), *S. typhi* (22.22%), *S. pneumonia* (27.58%) and *E. aerogens* (31.03%) and no activity against *S. aureus* and *K. pneumoniae*. The n-hexane fraction was significantly active against *K. pneumoniae* (66.66%) and moderately active against *E. coli* (48.14%), *S. typhi* (51.85%), *P. aeruginosa* (51.85%) and *B. pumalis* (40%). It showed low activity against *S. epidermidis* (34.61%), *S. pneumoniae* (20.68%), *S. aureus* (38.46%) and *E. aerogens* (34.48%). Chloroform fraction was moderately active against *K. pneumoniae* (57.14%), *S. typhi* (48.14%), *E .aerogens* (41.37%) and *B. pumalis (40%)* and low active against *S. aureus (38.46%), P. aerugenosa (37.03%), S. epidermidis (34.61%), E. coli (33.33%)* and *S. pneumoniae (24.13%).* Significant activity was shown by the*EtOAc*fraction against *K. pneumoniae (61.90%),* moderate against *S. typhi (48.14%), E. coli (44.44%), P. aeruginosa (44.44%)* and *B. pumalis (40%)* while low activity against *E. aerogens (37.93%), S. aureus (34.61%), S. epidermidis (34.61%)* and *S. pneumoniae (24.13%).* The aqueous fraction showed moderate activity against *S. epidermidis (53.84%), B. pumalis (44%), E. coli (48.14%)* and *S. typhi (40.74%),* low activity against *K. pneumoniae (38.09%), P. aeruginosa (37.03%), E. aerogens (37.93%), S. aureus (34.61%)* and no activity against *S. pneumoniae.* The n-hexane and EtOAc fractions exhibited significant while $CHCl_3$fraction showed moderate activity against *K. pneumoniae.* The aqueous fraction showed low activity against the majority of test pathogens.

Potency of a drug is inversely related to its MIC50 value. The MIC50 values of the test samples are presented in Table 2 and Figure 2. These values were calculated from three separate readings using Microsoft XL sheet. The MIC50 of the test samples range from 2.4-3.9 mg / ml.This work is continuation of my effort on exploring new bioactive compounds. In my previous work, I have utilized similar approach and explored various fractions of *Onosma griffithii* for antibacterial activity against *E. coli, B. subtilis, S. aureus, Shigella flexenari and S. typhi* (Ahmad et al., 2009). Same strategy was followed by Ajaiyeoba (2002), in which the n-hexane, ethyl acetate, ethanol and water extract of Parkia bicolor A. Chev were tested for antimicrobial activity against *S. aureus, B. cereus, E. coli, P. aeruginosa,* (Ajaiyeoba, 2002). The crude methanolic extracts of different plants were tested against gram positive and negative bacteria for new bioactive compounds (Shahidi, 2004).

CONCLUSION

The findings of the present study revealed that n-hexane, ethyl acetate extracts of *Acacia modesta* possess antibacterial activity.The n-hexane fraction was

significantly active against *K. pneumoniae* (66.66%) and moderately active against *E. coli* (48.14%), *S. typhi* (51.85%), *P. aeruginosa* (51.85%) and *B. pumalis* (40%). Significant activity was shown by the *EtOAc*fraction against *K. pneumoniae (61.90%),* moderate against *S. typhi (48.14%), E. coli (44.44%), P. aeruginosa (44.44%)* and *B. pumalis (40%)* So the research should be extended for the isolation of active compounds.

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Conflict of interests

Author have declared that no conflict of interests exist.

REFERENCES

1. Atta-ur-Rahman S.M., Ahmad V.U. Pakistan Encylcopaediaplantamedica vol. I and II. Hamdard Foundation Press, Hamdard Centre, Karachi, Pakistan, 1986.
2. Chopra R.N., Nayar S.L., Chopra I.C. Glossary of Indian medicinal plants. New Delhi. C SIR. 1956; 84: 9.
3. Lewis W.H., Elvin-Lewis M.P. Medical botany: plants affecting human health. John Wiley & Sons, 2003.
4. Nadkarni K.M. Indian materiamedica with ayurvedic, unani-tibbi, siddha, allopathic, homeopathic, naturopathic and home remedies, appendices and indexes. Bombay, Popular Prakashan, 1976: 278-79.
5. Asghar R., Ahmad M., Zafar M., Akram A., Mahmood J., Hassan M. Antibacterial Efficacy of Acacia modesta Wall (Miswak) against Dental Pathogen. Pakistan Journal of Pharmacological Sciences, 2003; 6(24): 2024-25.
6. Hussain F., Badshah L., Dastagir G. Folk medicinal uses of some plants of South Waziristan, Pakistan. Pakistan Journal of Plant Sciences, 2006; 12(1): 27-39.
7. Mahmood T., Khan M.A., Ahmad J., Ahmad M. Ethnomedicinal studies of Kala Chitta hills of district Attock, Pakistan. Asian Journal of Plant Sciences, 2004; 3(3): 335-39.
8. Qureshi R.A., Ahmed M., Ghufran M.A. Indigenous knowledge of some important wild plants as a folk medicines in the area of Chhachh (Distt. Attock) Punjab, Pakistan. Electronic Journal of Environmental, Agriculture and Food Chemistry, 2007; 6(11): 2500-11.
9. Zabihullah Q., Rashid A., Akhtar N. Ethnobotanical survey of KotManzary Baba valley, Malakand Agency, Pakistan. Pakistan Journal of Plant Sciences, 2006; 12: 115-21.
10. Ali S.I. Mimosaceae. Flora of West Pakistan, 1973.
11. Hook F. Acacia modesta. Flora of British India, 1878; 2: 296.
12. Ahmad B., Ali N., Bashir S., Choudhary M.I., Azam S., Khan I. Parasiticidal, antifungal and antibacterial activities of Onosmagriffithii Vatke. African Journal of Biotechnology, 2009; 8(19): 5084-87.
13. Rios J.L., Recio M.C., Villar A. Screening methods for natural products with antimicrobial activity: A review of the literature. Journal of Ethanopharmacology, 1988; 23(2): 127-49.
14. Ahmad B., Khan I., Azam S., Bashir S., Ahmad J., Hussain F. Screening of Acacia modesta for haemagglutination, antibacterial, phytotoxic and insecticidal activities. Journal of Medicinal Plants Research, 2011; 5(14): 3090-96.
15. Napar A.A., Bux H., Zia M.A., Ahmad M.Z., Iqbal A., Roomi S., Muhammad I., Shah S.H. Antimicrobial and antioxidant activities of Mimosaceae plants; Acacia modesta Wall (Phulai), Prosopis cineraria (Linn.) and Prosopisjuliflora (Swartz). Journal of Medicinal Plants Research, 2012; 6(15): 2962-70.
16. Jawla S., Kumar Y., Khan M.S.Y. Antimicrobial and antihyperglycemic activities of Acacia modesta leaves. Pharmacologyonline, 2011; 2: 331- 47.
17. Khalid A., Rehman U., Sethi A., Khilji S., Fatima U., Khan M.I. Mahmood, S. Antimicrobial activity analysis of extracts of Acacia modesta, Artimisiaabsinthium, Nigella sativa and Saussurealappa against Gram positive and Gram negative microorganisms. African Journal of Biotechnology, 2013; 10(22): 4574-80.
18. Rashid A., Hashmi H. In vitro Susceptibility of some gram positive and gram negative strains of bacteria and fungi to root extracts of Acacia modesta. Pakistan Journal of Pharmacological Sciences, 1999; 2(3): 746- 49.
19. Tirillini B., Velasquez E.R., Pellegrino R. Chemical composition and antimicrobical activity of essential oil of Piper angustifolium. PlantaMedica, 1996; 62(4): 372-73.
20. Khan I. Phytochemical Evalution, Bioassay Screening and Standardization of Zizyphusjujuba and Acacia modesta. PhD. Thesis, University of Peshawar, Khyber Pakhtunkhwa, Pakistan, 201.