

Phytochemical Investigation and Biological Analysis of Plant *Acacia Modesta*

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Abstract: *The purpose of the present investigation was to ascertain the effectiveness of the drugs under review. As a result, standardizing medicines requires the execution of many parameter checks. For example, to demonstrate the diagnostic capabilities of plants, phytochemical screening of recently made drugs has to be done. Furthermore, this is used for biological activities including antifungal and antibacterial tests, which is highly suggestive for further evaluation in the form of in vivo and ex vivo investigations and a strong premise for clinical trials as well. Studies provide insight on the introduction and proper use of traditional remedies in contemporary times. Researchers have tried to show the biological and chemical potential of the medicinal plants based on studies of the literature*

Keywords: Phytochemical Investigation

I. INTRODUCTION

The genus *Acacia*'s plants are known by their vernacular name, *Acacias*. They belong to the family *Mimosaceae*. The name *Acacia* comes from the Greek word "akis," which meaning "sharp point". The *Acacia* genus of plants is resistant to extreme drought and xerophytic conditions. On sandy, salinized, and even damp soils, they may thrive. This family's members are seldom plants, shrubs, or trees; instead, they are often spinose or thorny. The leaves are bipinnate and have a few, spiny, membranous stipules that are scarce. Leaflets are typically quite little, although they may become multijugate or a petiole (phyllode) that resembles a leaf. Small, globose heads or cylindric spikes with axillary, solitary, fascicled, or paniced peduncles at branch ends; hermaphrodite or polygamous flowers, usually five in number. Calyx campanulate, often known as the short-toothed funnel. Petals are usually connected, seldom free, and exerted. Stamens are ambiguous, extremely extended, and free, whereas anthers are small and lack glands. Filiform style, many ovules, sessile or stalked ovary, and a small, terminal stigma. The pod may be subcylindric, ligulate, or oblong, without joints, often dry and compressed, dehiscent or in-dehiscent, seldom turgid, and have straight or wavy, non-thickened sutures. The seeds are surrounded with filiform funicle or large, fleshy arils [1].

Many purposes have been assigned to *acacia* trees [2]. One of the best sources of charcoal is the wood of the *Acacia* tree, which is also useful for making train carriages, wheels, and furniture [3]. Humans eat the seeds and pods of several *Acacia* species. Tanaka [4] discovered 56 species of edible *acacias*. *Acacia* tree honey is highly valued for its sweetness and non-crystallization. The *Acacia* species is well known for its popular gums. Gum-producing species include *Acacia arabica*, *Acacia catechu*, *Acacia churnea*, *Acacia farnesiana*, *Acacia jacquemontii*, *Acacia leucophloae*, *Acacia modesta*, *Acacia senegal*, and *Acacia auriculiformis* [5]. Gum *Arabica* is used in plastic surgery to alleviate low blood pressure brought on by bleeding or surgical shock, as well as to repair damaged peripheral nerves [6]. The bark of *A. farnesiana* and the leaves of *A. concina* are used to cure malaria [7]. Parkiabicolor *A. Chev's* n-hexane, ethanol, ethyl acetate (EtOAc), and water extract all exhibited concentration-dependent antibacterial activity [8]. With minimum bactericidal concentrations (MBC) of 35 and 60 mg/ml and minimum inhibitory concentrations (MIC) of 35 and 50 mg/ml, *A. nilotica* stem bark extracts have shown antibacterial effectiveness against *Escherichia coli*, *Shigellasonnei*, *S. aureus*, and *Streptococcus viridans* [9].
Supplies and Methods

II. PHYTOCHEMICAL SCREENING

Plant material

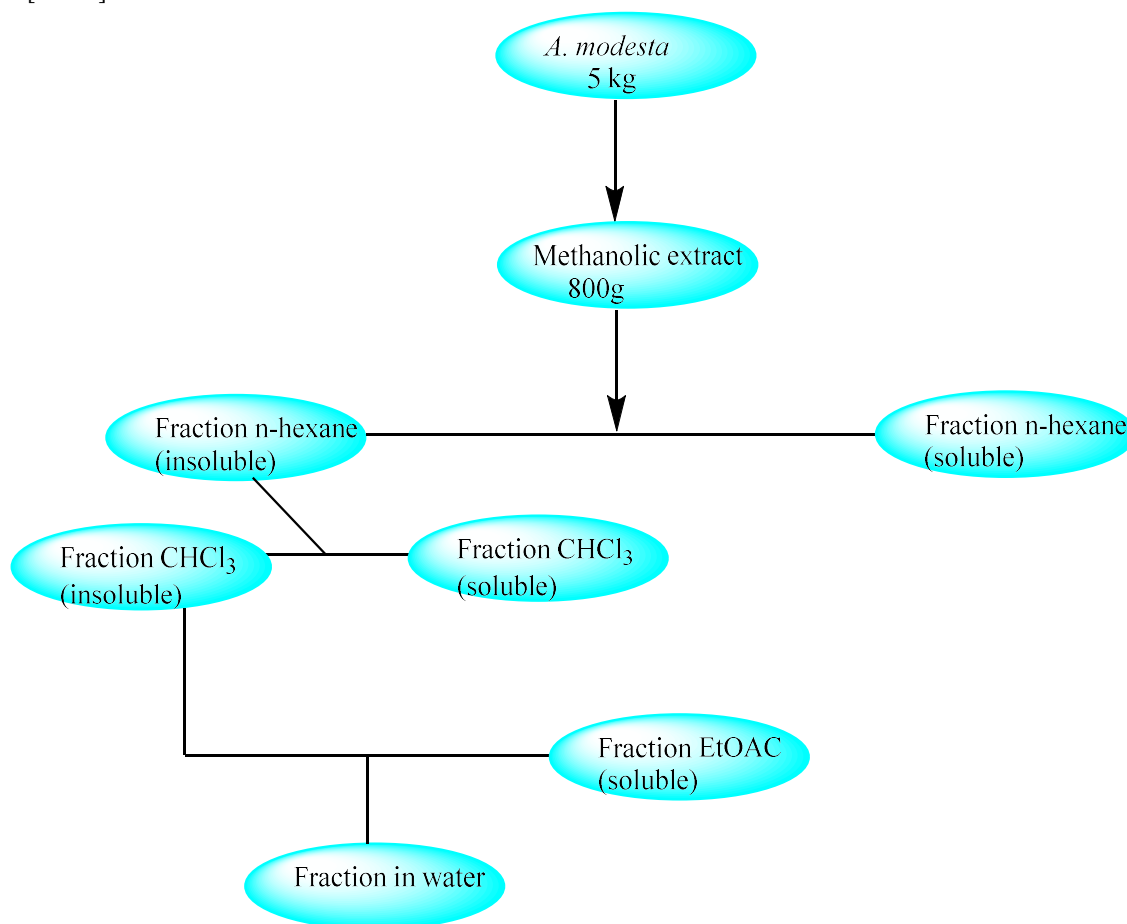
The northern region of India is home to *A. modesta*, which is mostly found there in the winter.

Extraction

The plant material was kept in the shade to dry. After they had dried, they were chopped into minuscule bits and ground into a powder using an electric grinder. Five kilograms of the powdered substance were steeped in commercial-grade methanol twice for a duration of twenty days at room temperature, with occasional shaking. Each time the material was shown. 800 g of blackish crude methanolic extract were produced when all the filtrates were combined and vacuum-concentrated below 40 °C [10–11].

Fractionation

The crude methanolic extract (800 g) was diluted in 400 ml of distilled water, and then divided into three equal parts using n-hexane (3 x 500 ml), CHCl₃ (3 x 500 ml), and EtOAc (3 x 500 ml). This resulted in the following fractions: n-hexane (240 g), CHCl₃ (200 g), EtOAc (50 g), and aqueous (370 g), respectively. Fig. 1 and Scheme 1 show the fractionation scheme. For use in biological and pharmacological research, 90 g of the crude methanolic extract were set aside [12–13].



ANTIBACTERIAL ACTIVITY

Infection incidence is now on the increase as a result of the introduction of new pathogens and the resistance of established diseases to medications. For instance, isoniazid and rifampicin are no longer effective against multi-drug resistant tuberculosis (MDR-TB). Plants are rich in bioactive compounds. Twelve of the top 25 pharmacological agents marketed globally are from natural products. Consequently, the current study aims to discover new antibacterial chemicals derived from plants.

Materials

The test organisms used in the current study for crude methanolic extract and various fractions were *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Bacillus Pumilus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Streptococcus pneumonia*. The test organisms used for oils were *E. Coli*, *Shigella flexenari*, *Bacillus subtilis*, *S. aureus*, *P. aeruginosa*, and *S. typhi*. Analytical-grade organic solvents were used in the research. Other materials utilized were a 6mm borer, Petri plates, an autoclave, micropipettes, an incubator, standard antibiotic (Amoxicillin), dimethyl sulfoxide (DMSO), test samples (crude methanolic extract and plant fractions), and an autoclave.

Procedure

The following methods were used to assess the test samples' antibacterial efficacy against the pathogens listed above. The experiment was carried out according to the provided procedure. To verify sterility, nutrient agar medium and nutrient broth were made and cultivated for 24 hours at 37 oC[14]. A loop dipped in the bacterial culture-containing broth was moved to new broth for dilution on the second day. In one milliliter, this broth culture was shaken to create a bacterial lawn on nutrient agar plates (milliliter). For the nutritional agar plates, wells were made and labeled using a 6mm borer. To make stock solutions, one milliliter of DMSO was combined with three milligrams of the test samples. 100µl of stock solutions were added to each well, and the wells were then incubated for 24 hours at 37 oC. Amoxicillin served as the positive control, while DMSO served as the negative control. The next day, the zone of inhibition was assessed. Using the following formula [15], the percent zone of inhibition was calculated with respect to the positive control.

$$\% \text{ Inhibition} = \frac{\text{Zone of inhibition of sample}}{\text{Zone of inhibition of standard}} \times 100$$

III. RESULTS

PHYTOCHEMICAL INVESTIGATIONS

Looking for Different Compound Groups Numerous naturally occurring substances present in plants, including tannins, saponins, alkaloids, and flavonoids, are closely linked to the health benefits of that particular plant. These natural substances are isolated, then synthesized in various pharmaceutical dosage forms to treat a variety of illnesses. A. modesta was investigated to see whether any of the many types of natural products were present. Table 1's data indicates that precipitate development was seen during the alkaloids test, indicating the presence of alkaloids in this plant. The development of a pink tint in the flavonoid test indicates that the plant contains the flavonoid group of natural substances. The saponin presence result was negative since no foam developed. The formation of the precipitate and the subsequent appearance of a blue-green hue indicated the presence of tannins in the plant.

Table 1 Different chemical test performed and there results

S. No.	Performed Test	Result
1	Alkaloids	Positive
2	Flavanoids	Positive
3	Tanins	Positive
4	Saponins	Negative

ANTIBACTERIAL ACTIVITY

Fractions and crude methanolic extract. One of the most significant issues facing medical research today is antibiotic resistance. Developing inventive and imaginative antimicrobials will assist us in addressing this issue. *Staphylococcus aureus* used to be sensitive to many medications, but it has now developed resistance to them. It has developed resistance to many antibiotics, including gentamicin, pencillin G, macrolides, lincosamides, and tetracyclines. The purpose of evaluating the test materials against certain illnesses was to discover novel bioactive chemicals originating from plants. Antibacterial activity data are shown in Table 2 and Fig. 2-5. The crude methanolic extract had no impact against *S. aureus* and *K. pneumoniae*, while it was somewhat effective against *E. coli* (40.64%), *P. aeruginosa* (40.64%), and *B. pumalis* (40%). The effectiveness of the medication was shown to be poor against *S. pneumoniae* (27.48%), *S. typhi*

(22.12%), *S. epidermidis* (34.51%), and *E. aerogens* (31.13%). The n-hexane fraction shown high activity (66.56%) against *K. pneumoniae*. However, its effectiveness against *B. pumalis* (40%), *S. typhi* (51.75%), *P. aeruginosa* (51.75%), and *E. coli* (48.04%) was rather average. There was little action shown against *S. aureus* (38.36%), *S. pneumoniae* (20.58%), *S. aerogens* (34.38%), and *S. epidermidis* (34.51%). In comparison to *S. aureus* (38.36%), *P. aeruginosa* (37.13%), *S. epidermidis* (34.51%), *E. coli* (33.23%), and *S. pneumoniae* (24.03%), the chloroform fraction was shown to be moderately active against *K. pneumoniae* (57.04%), *S. typhi* (48.04%), *E. aerogens* (41.27%), and *B. pumalis* (40%). The EtOAc fraction exhibited notable activity against *K. pneumoniae* (61.80%), but only moderate activity against *S. typhi* (48.04%), *E. coli* (44.34%), *P. aeruginosa* (44.44%), and *B. pumalis* (40%). Additionally, it had little action against *S. aureus* (34.51%), *S. epidermidis* (34.51%), *S. pneumoniae* (24.03%), and *E. aerogens* (37.83%). The aqueous fraction exhibited weak activity against *K. pneumoniae* (38.09%), *P. aeruginosa* (37.13%), *E. aerogens* (37.83%), and *S. aureus* (34.51%) and no activity against *S. pneumoniae*. The aqueous fraction showed moderate efficacy against *S. epidermidis* (53.74%), *B. pumalis* (44%), *E. coli* (48.04%), and *S. typhi* (40.64%). The majority of the test pathogens are not efficiently inhibited by crude methanolic extract, as the findings previously mentioned suggest. While the CHCl₃ fraction only had modest activity against *K. pneumoniae*, the n-hexane and EtOAc fractions shown substantial activity.

Table 2 Antibacterial activity of the different fractions

Bacteria	Zone of inhibition(standard)	Methanolic extract		n-hexane		CHCl ₃		EtOAc		Aqueous	
		Zone of inhibition	% inhibition	Zone of inhibition	% inhibition	Zone of inhibition	% inhibition	Zone of inhibition	% inhibition	Zone of inhibition	% inhibition
<i>E. coli</i>	27	11	40.64	13	48.04	9	33.23	12	44.34	13	48.04
<i>S. epidermidis</i>	27	9	34.54	9	34.51	9	34.51	9	34.51	14	53.64
<i>S. typhi</i>	28	6	22.12	14	51.75	13	48.04	13	48.04	11	40.64
<i>S. pneumoniae</i>	28	8	27.48	6	20.58	7	24.03	7	24.03	0	0
<i>S. aureus</i>	26	0	0	10	38.36	10	38.54	9	34.51	9	34.51
<i>P. aeruginosa</i>	25	11	40.64	14	51.75	10	37.13	12	44.34	10	37.13
<i>K. pneumoniae</i>	21	0	0	14	66.56	12	57.04	13	61.80	8	38.19
<i>B. pumalis</i>	25	10	40	10	40	10	40	10	40	11	44
<i>E. aerogens</i>	29	9	31.13	10	34.38	12	41.27	11	37.83	11	37.83

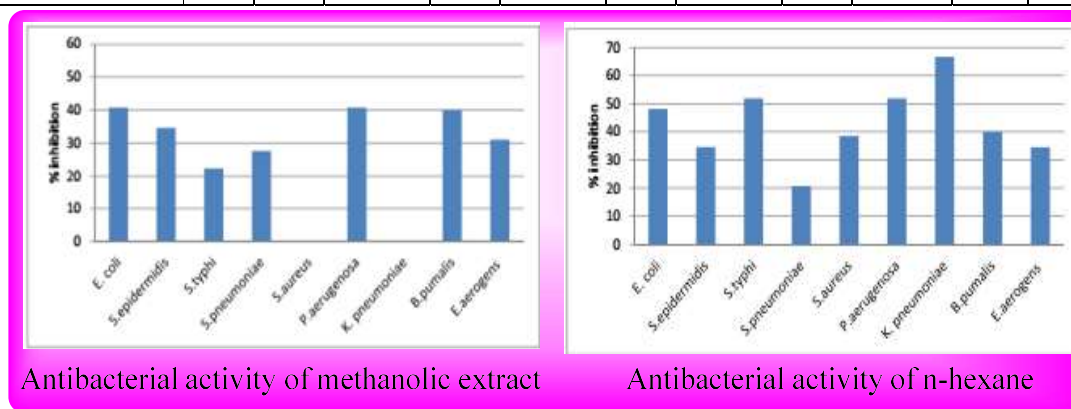


Fig 2 Antibacterial activity as recorded in methanolic extract and n-hexane

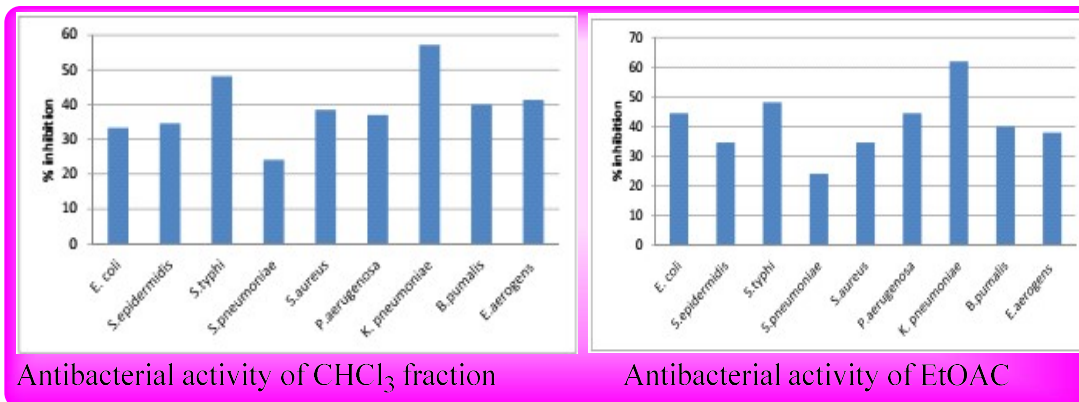


Fig 3 Antibacterial activity as recorded in CHCl₃ and EtOAc

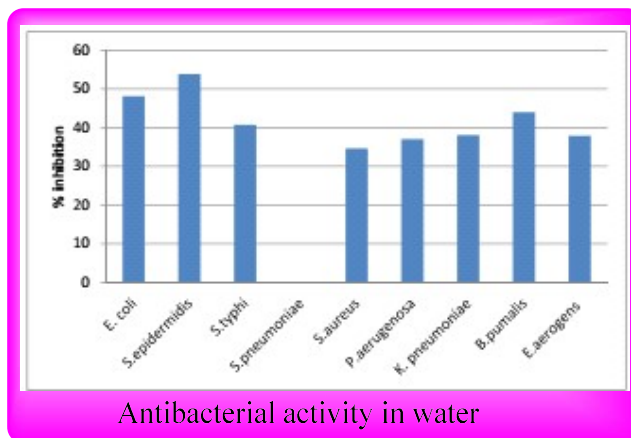


Fig 4 Antibacterial activity as recorded in aqueous

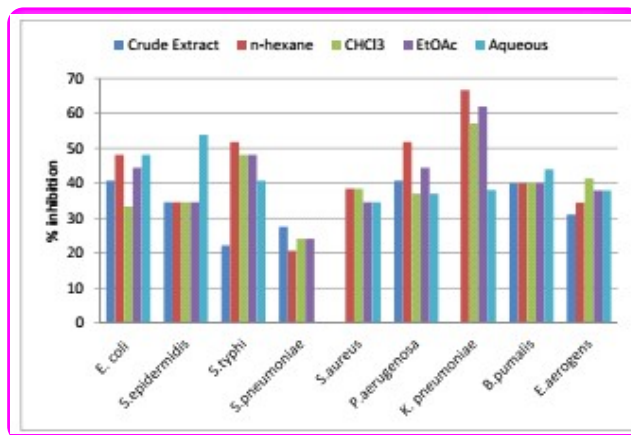


Fig 5 Combined antibacterial activity as recorded in different fraction

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