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PHYTOCHEMICAL AND ANTIBACTERIAL POTENTIAL OF ACETONE LEAF EXTRACT OF *ACACIA CONCINNA (WILLD.)* DC.

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ABSTRACT: The present study was aimed to find out the antimicrobial potential of acetone extract of *Acacia concinna* leaf against five different bacterial strains at 100 and 200mg/ml concentrations. The disc diffusion assay was performed to access the antimicrobial potential of the extract. The agar plate containing a bacterial culture was divided into 4 zones and each zone was independently impregnated with four sterile filter paper discs (6mm in diameter) containing (10µl of each concentration) distill water (Negative control), ampicillin (Positive control); *Acacia Concinna* leaf extract (100mg/ml and 200mg/ml as Test). Zone of inhibition and MIC (minimum inhibitory concentration) for test drug was evaluated to access the antimicrobial potency. The phytochemical analysis shows the presence of alkaloids, tannins and glycosides that might be responsible for the aimed biological activities. Out of five bacterial strains, the test drug exhibit zone of inhibition against *Mycobacterium smegmatis* and *Bacillus subtalis* in concentration dependent manner. Minimum inhibitory concentration (MIC) of test drug against *Mycobacterium smegmtis* and *Bacillus subtilis*, was found to be 2.5 (mg/ml) and 1.25 (mg/ml) respectively. The basis of above findings suggests the need for further future perspective works on the plant extract of *Acacia Concinna* which might be very useful as it seems to be a potential source for arresting the growth and metabolic activities of strains like Mycobacterium smegmtis and *Bacillus subtilis*.

INTRODUCTION: Medicinal plants have been identified used by mankind since time immemorial [1]. Plants have the ability to synthesize a wide variety of chemical compounds that perform important biological functions [2]. India is a country with a vast reserve of natural resources and a rich history of traditional medicine [3]. Medicinal plants contain numerous biologically active compounds which are helpful in improving the human life [4]. The traditional medical system, especially the use of medicinal plants, still plays a vital role to cover the basic health needs in the developing countries [5].

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India is rich in the medicinal herbs and therefore, it can be accurately called the "Botanical Garden of the World" [6]. *Acacia concinna* is widely distributed in Burma, southern China and Malaysia. It is found in central India commonly in Madhya Pradesh, Maharashtra and in some regions of pinnae. The leaf stalks are 1.5cm long with a prominent gland about the middle longer. It is a climbing shrub with thorny branches having brown smooth stripes. Thorns are short, broad-based, flattened surface. The main stem is brown in color. The stem is strong, woody and armed.

The barks present on the stem have longitudinal striations. The bark is generally thick and rough. The stem is dotted with white dots on its bark. Its bark contains thorns on its surface. The girth of the main stem is generally 16-20cm.Leaves have characteristic odor and taste. Flowers are 1.5-2 cm

long. They are round to oblong shape. They have a diameter of 1cm. They are pink in color. They are bisexual, mostly funnel shaped. The flowers are stalked, complete, bracteate, regular, actinomorphic, pentamerous, and hypogynous. Inflorescence is terminal cyme, each bearing 60 to 90 flowers. Calyx is five lobed and green in color. Corolla is white in color and is inferior with five petals. Stamens are inferior and about 10 in number. The female part is 5 to 6 mm long. Stigma is bright and sticky. Style is short and ovary is superior. Fruits are known as legumes and these are fleshy, beaked and constricted. There are 6-10 seed in each fruit. The seeds are brown in colour. The pod is about 5-6 cm [7-10] *Acacia concinna* (Shikakai) grows in tropical forest of India. Its fruits are very well known for use as natural hair shampoo. Its pods turn dark brown and wrinkled on drying. For medicinal purposes its leaves and leaves and fruits are used.

Its dried pods are powdered to produce shikakai powder. Shikakai is also used in traditional medicine to treat jaundice, constipation and skin problem, itching, pimples, hyperpigmentation, bad, leprosy, psoriasis and gum infection, Dandruff, Leprosy, Psoriasis [11-12]. Now-a-days there are many major problems that the world is facing among of them the microbial resistance against the existing antibiotics, which is of great concern and this issue have necessitated the search for new antimicrobials [13]. More and more about plants, therefore, are to be screened to find out their therapeutic potential. Keeping in view these issues, in the present investigation, we have selected *Acacia concinna* (**Fig.1, Table 1**) for evaluating its phytochemical and antibacterial properties as it has been traditionally used for the treatment of various human ailments [14]. The antibacterial properties have been studied against five pathogenic bacteria using disc diffusion and MIC assay. The plant is known by different names in different part of the country and the world.

The names *acacia* is derived from the Greek word "akis" meaning "sharp point" [15] and relates to the sharp thorny shurbs and tree of tropical Africa and Western Asia that were only known acacias at the time that the name was published [16]. The Australian *acacias* are commonly called "wattles" because of their pliable branches that were woven into the structure of early wattle house and fences [17]. Keeping in view the importance of plants for the Benefit of mankind, phytochemical and antimicrobial potential of *Acacia concinna* was studied in the present investigation.

MATERIALS AND METHODS:

Microbial assay:

Preparations of plant extract: [18]

The leaves were collected in the month of January from the herbal garden of the Maharshi Dayanand University (MDU), Rohtak. The leaves were washed and shaded dry at room temperature followed by oven drying at 30-35˚C. The dried leaves were pulverized and were further used for extraction. The powdered plant material was extracted in acetone using soxhlet apparatus.

Test organism:

The bacterial strains used for assessing the antibacterial potential of the plant material were procured from Institute of Microbial Technology, CSIR, Chandigarh. Bacterial strains used in the present studies were *Pseudomonas auregiuosa (2453), Klebsella pneumonia (109), Mycobacterium smegmatis (992), Staphylococcus aureus (96) and Bacillus Subtilis (2657)*

Phytochemical screening:

Acetone extract of *Acacia concinna* leaf was subjected to various phytochemical tests for the identification of phytoconstituents present therein. [19-23]

Disc diffusion assay: [24]

Disc of whatman filter paper (6mm diameter) were prepared and the bacterial strains were revived by inoculating in broth media. Incubation was carried out at 37ºC for 18 hrs for bacterial growth. The agar plate containing a bacterial culture was divided into 4 zones and each zone was independently impregnated with four sterile filter paper discs (6mm in diameter) containing (10µl of each concentration) distill water (Negative control), ampicillin (Positive control); *Acacia Concinna* leaf extract (100mg/ml and 200mg/ml as Test) and were air dried to eliminate any residual solvent and were placed on their respective zone on the agar plate containing microbial strain. The plates were

incubated for 24hrs at 37˚C in a B.O.D. incubator after that, diameter of the inhibition zones obtained was measured.

Minimum inhibitory concentrations (MIC) assay [25-29]:

MIC was determined by micro broth dilution technique using serially diluted (2 fold) plant extracts with little modifications. Equal volume of each extract and nutrient broth were mixed well. Specifically equal amount of standardized inoculum (1×10^7cfu/ml) and resazurin sodium salt indicator were added in each well. The plates were incubated at 37 °C for 24 h. The negative control used was broth media, resazurin sodium salt indicator and bacterial inoculum. The lowest concentration (highest dilution) of the extract that produced no color change (purple to pink) when compared with the control was regarded as MIC for those particular bacteria.

$$\% \text{ Inhibition} = 100 - \left[\frac{\text{OD of culture with sample (Test)}}{\text{OD of culture without sample (Control)}} \times 100 \right]$$

RESULTS:

Phytochemical Analysis:

The results for the phytochemical analysis obtained are depicted below in **Table 1**. The result showed that acetone extract of the leaf *Acacia concinna* leaf contains alkaloids, tannins and glycosides.

TABLE 1: QUALITATIVE ANALYSIS OF PHYTOCHEMICAL SCREENING OF ACETONE EXTRACT OF *ACACIA CONCINNA*

Phytochemical constituents	Result
Flavonoids	-
Alkaloids	+
Tannins	+
Saponins	-
Glycosides	+

+ sign indicates the presence of phytochemical constituents and – sign indicates the absence of phytochemical constituent.

Determination of zone of inhibition by disc diffusion assay:

Out of five strain, *Mycobacterium smegmatis* and *Bacillus subtalis* showed zone of inhibition at the concentration of 200 mg/ml were 12mm & 10mm and minimum at 100mg/ml were 10mm & 8mm respectively and control drug (Ampicillin) showed 9.3 mm & 9mm respectively, but distill water did not show any zone of inhibition in both bacteria.

When the acetone leaf extract was tested against *Klebsiella pneumoniae, Pseudomonas auregiuosa*, S*taphylococcus aureus* no zone of inhibition was observed with all the concentration of extracts used, but the positive control exhibit 7.5 mm, 9 mm & 1cm respectively zone of inhibition but against *Mycobacterium smegmatis* and *Bacillus subtalis*, zone of inhibition was observed, maximum zone of inhibition was shown at the concentration of 200 mg/ml were 12mm & 10mm and minimum at 100mg/ml were 10mm & 8mm respectively and control drug (Ampicillin) showed 9.3 mm & 9mm respectively, but distill water did not show any zone of inhibition in both bacteria.

TABLE 2: ANTIMICROBIAL EFFECT OF *ACACIA CONCINNA* ON ZONE OF INHIBITION AGAINST DIFFERENT BACTERIAL STRAINS

Pathogens	Treatment			
	***Acacia concinna* (100mg/ml)**	***Acacia concinna* (200mg/ml)**	**Positive control Ampicillin (1µg/ml)**	**Negative control (Broth Media)**
Pseudomonas auregiuosa	Nil	Nil	9.0mm	Nil
klebsilla pneumonia	Nil	Nil	7.5mm	Nil
Staphylococcus aureus	Nil	Nil	10mm	Nil
Bacillus subtilis	8mm	10mm	9mm	Nil
Mycobacterium smegmatis	10mm	12mm	9.3mm	Nil

Determination of minimal inhibitory concentration (MIC) by Micro Broth Dilution Technique:

The leaf extract of *Acacia concinna* was ananlysed to find out the minimum inhibitory concentration against *Mycobacterium smegmtis* and *Bacillus subtilis* which exhibited maximum activity by disc diffusion assay among all the pathogens selected in the study. The results of MIC are given below in **Table 3.**

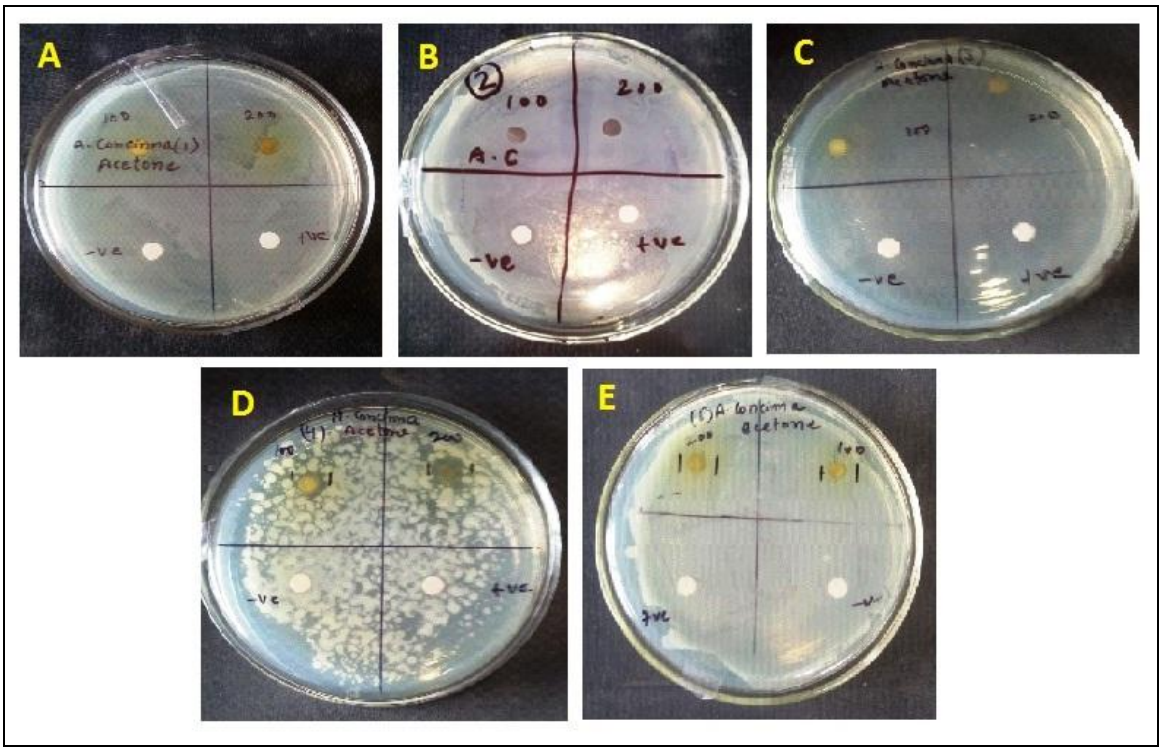

FIG.1: ZONE OF INHIBITION WHEN PLANT EXTRACT WAS TESTED AGAINST (A) *KLEBSIELLA PNEUMONIAE* (B) *PSEUDOMONASAUREGIUOSA* (C) *STAPHYLOCOCUS AUREUS* (D) *MYCOBACTERIUM SMEGMATIS* (E) *BACILLUS SUBTALIS*

TABLE 3: MIC RESULTS AGAINST *MYCOBACTERIUM SMEGMATIS.*

Sr. No.	Microorganism	MIC (mg/ml)
1.	*Mycobacterium smegmatis*	2.5
2.	*Bacillus subtilis*	1.25

DISCUSSION AND CONCLUSION:

The present study was carried out to find out the phytochemical and the antibacterial potential of the leaves of *Acacia concinna* extracted with acetone solvent**.** The extract was analyzed qualitatively for the presence of various phytochemical compounds and alkaloids, tannins and glycosides were found to be present in the extract. The screening of secondary Metabolites has shown that higher plants represent a potential source of new anti- infective agents [30-33]. Earlier studies on phytochemical investigations on the leaf extract of *Acacia concinna* reported the presence of terpenoides [34-35] saponins [36], tannins [37]. Johnson et al., 2005 and Raja, et al., (2011) also analyzed the leaf extract phytochemically and reported the presence of alkaloids and flavonoids, respectively [38-39]. However, in the present study, flavonoids and sapononis were found to absent in the leaf extract. This is probably due to the collection of plant material from different geographical zone. In addition, the solvent used might also restrict the release of some phytocompounds during extraction. In this Study, maximum activity was observed against *Mycobacterium smegmatis*, followed by *Bacillus subtilis.* The present study thus supports that the plants possess antibacterial potential. Similar to my results, earlier research also reported the antibacterial activity of leaf extract [37].

Some of the research has also reported the antifungal activity of the Leaf extract. Ethanolic leaf extract of *Acacia concinna* has been shown to exhibit maximum activity against *Aspergillus niger* [37] followed by *Penicillium species* [39] and *Candida albicans* [37].

Plant extracts have assumed increased importance In healthcare industry due to their biological properties. In this study, we used only the crude leaf extract of *Acacia concinna.* The study can be extended further by identifying the active

compound and compare the activity with known antimicrobial agents. The study should be carried out with other pathogens also like pathogenic fungi. In addition to acetone more solvents can be used for investigation the biological properties of the plant. Thus, further future perspective works on the plant extract of *Acacia concinna c*an be very useful as the plant seems to be a potential source for arresting the growth and metabolic activities of bacterial and fungal strains.

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