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ANTIMICROBIAL PROPERTIES AND PHYTOCHEMICAL EVALUATION OF *ACACIA NILOTICA* FROM DIFFERENT ZONES IN INDIA.

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ABSTRACT

Phytochemical quantification and analysis of antimicrobial activities of *Acacia nilotica* from 12 different locations in Bihar, Uttar Pradesh and Madhya Pradesh were performed against *E. coli, P. aeruginosa, S. aureus* and *B. subtilis*. Our data suggests that out of all eight phytochemicals tested, bark of the plant contained variety of compounds in comparison to leaves. Out of our 12 samples, almost all of the plant extracts were found effective against all of the pathogens selected in the study.

KEYWORDS: ***Acacia nilotica*, antimicrobial properties, pathogens, *E. coli, S. aureus*.**

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INTRODUCTION

A number of medicinal plants are gifted by nature to mankind and a notable number of modern drugs have been synthesized from these natural medicinal plants. These drugs are based on the indigenous medicinal information on plants. This natural source has been used to cure various diseases throughout the world. Actually, plants have great diversity of bioactive compounds and it is an indication which makes plants a prosperous source of different types of drugs (Mohammad, *et. al*., 2012). *Acacia nilotica* is a pioneer species, relatively high in bioactive secondary compound and are important for a variety of functions is economically used as a source of tannins, gums, timber, fuel and fodder. It is commonly known as '*Babul*' plant having various therapeutic uses such as: anti-cancer, anti tumour, antiscorbutic, astringent, anti-oxidant, antispasmodial, diuretic, intestinal pains and diarrhoea, nerve stimulant and is used for treatment of cold, congestion, coughs, dysentry, fever, hemorrhages, leucorrhea, ophthalmia and sclerosis (Solomon-Wisdom & Shittu, 2020; Malviya, *et. al*., 2011). Number of plants have been studied and screened against pathogenic organisms and showed prominent antimicrobial activities *in-vitro* and *in-vivo*, which justifies that indigenous medicines be focused on characterization of antimicrobial activities of different plants. Plant extracts and photochemical compounds have been used for antimicrobial properties and have significant therapeutic properties. In recent years, various investigations have been conducted worldwide to confirm such efficiency. Various plants showed antimicrobial properties, which were similar to that of synthetic standard antimicrobial agents. The potential of higher plants as source of new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and a fraction investigated for biological and pharmacological screening. Any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents (Mahesh & Satish, 2008).

MATERIALS AND METHODS

Collection and Preparation of the Plant Material

The leaves and bark of *Acacia nilotica* were collected from different locations of District Jhansi, Bundelkhand region, U. P. in November 2013. First leaves and bark part of plant washed with the distilled water then Leaves and bark parts of the plant materials were dried in an open air protected from direct exposure to sunlight. The identities of each plant specimen were confirmed at the National Herbarium, MRD LifeScience Laboratary, Lucknow. The dried plant materials were separately powdered for extraction with grinder.

Samples were labelled as below throughout the study:

Sample 1: Datia, (M. P.)
Sample 2: Vill. Barata, Distt. Jhansi, (U. P.)
Sample 3: Vill. Paricha, Distt. Jhansi, (U. P.)
Sample 4: Vill. Karguon, Distt. Jhansi, (U. P.)
Sample 5: Town Chirgaon, Distt. Jhansi, (U. P.)
Sample 6: Vill. Girgaon, Distt. Gwalior, (M. P.)
Sample 7: Vill. Girgaon, Distt. Gwalior, (M. P.)
Sample 8: Town Datia, Distt. Datia, (M. P.)
Sample 9: Darbhanga, (Bihar)
Sample 10: Vill. Muskara, Distt. Hamirpur, (U. P.)
Sample 11: Vill. Basbari, Distt. Hamirpur, (U. P.)
Sample 12: Town Rath, Distt. Hamirpur, (U. P.)

Preparation of Crude Extracts

10g of each sample (leaves and bark) dissolved in 100ml each solvent; such as methanol, acetone and ethyl acetate for maceration (Pandey & Kumar, 2011). Maceration was continued for 48 hours with frequent agitation and the resulting liquid is

filtered using filter paper (Whatman No 1, Whatman Ltd., England). Extraction was repeated five times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using rota vapor (BÜCHI Rota-vapor R-205, Switzerland) at not more than 40^0C. The aqueous residue was then placed in an oven at 40^0C for about 48 hours to remove the water. The resulting dried mass collected was preserved for further procedure.

Tested Microorganism

Bacterial culture were obtained from IMTECH, Chandigarh. Cultures were maintained by MRD LifeScience, Lucknow. Two Gram positive *Staphylococcus aureus* (MTCC 2940) and *Bacillus subtilis* and two Gram negative *E. coli* (MTCC 739) and *Pseudomonas aeruginosa* (MTCC 2453) were used.

Antibiogram analysis

The antibacterial activity of *Acacia nilotica* extracts was determined by agar well diffusion method against *S. aureus, P. aeruginosa, E. coli* and *B. subtilis* (Riaz, *et. al*., 2011; Irshad, *et. al*., 2012). MIC of the plant extracts were calculated as per methods described earlier by (Pandey and

Mishra, 2011)

Plant sample may contain some significant phytochemicals can be detected by using best solvent and extraction methods, chemical test are conducted on the aqueous extracts of each plant material and also the powdered form of plant as well as powdered extract of samples.

Phytochemical analysis

The leaves, peels and fruits extracts were screened for some secondary metabolites like-saponins, tannins, alkaloids, anthraquinones, phlobatannins, flavonoids, terpenoids, reducing sugar and poly phenols according to method reported earlier (Despande, 2013; Despande and Kadam, 2013; Sarkiyayi and Abdulrsheed, 2013; Wadood, et. al., 2013).

Test for reducing sugar : Take 1ml or 1gm of plant sample in a test tube and add 10ml deionized water then add few drops of Fehling solution (1ml Fehling solution A and B) and heat at 100^0C in a water bath. Brick red precipitate shows a positive result.

Test for tannins**:** Take 2gm of aqueous extract in a test tube and add 2 drops of 5% ferric chloride, brown color gives positive result.

Test for phlobatannins**:** Take 2ml plant sample in a test tube and add 10ml deionized water and boil at 100^0C with a few drops of 1% HCl. Deposition of red precipitation gives a positive result.

Test for Saponins**:** Saponin content is determined by boiling 1ml plant sample in 10 ml deionized water for 15 min. and after cooling the extract was shaken vigorously to record froth formation.

Test for terpenoids**:** Take 5ml of aqueous extract add 2ml chloroform followed by addition of 3ml conc. sulfuric acid, observe the reddish brown interface for presence of terpenoids.

Test for alkaloids**:** Take 1ml of aqueous extract in test tubes and add 2-3 drops of Wagners reagent it gives orange red precipitation.

Test for flavonoids**:** Add few drops of 1% NH_3 yellow colour observed, showed presence of flavonoids then after this take ethanolic or aqueous extract and add 10ml DMSO then heat it followed by adding Mg (magnesium chloride), add conc. HCl gives red color to confirmed flavonoids.

Test for polyphenols**:** Take 2ml ethanolic extract of plant sample and add 1ml Folin-Ciocalteu reagent and 9ml d/w. between 1-8 min. And add sodium carbonate solution (8ml) vortex to mix, then kept the test tube in the dark take OD at 760nm.

RESULTS

Phytochemicals in the plant extracts

The phytochemicals present in the plant materials were analyzed and were listed in table 1 below.

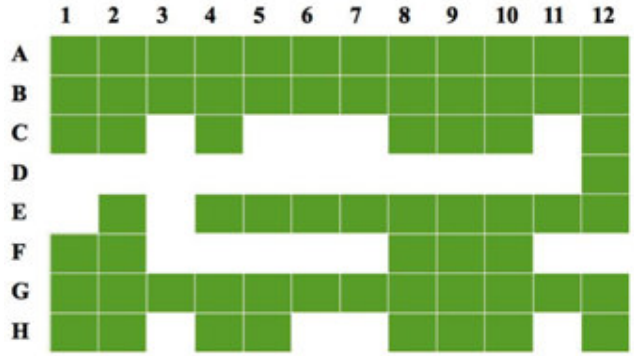

Figure 1

Photograph showing presence and absence of different types of phytochemicals in the leaves from of the 12 samples; A) reducing sugars, B) tannins, C) phlobatannins, D) saponins, E) terpenoids, F) alkaloids, G) polyphenols and H) cardiac glycosides.

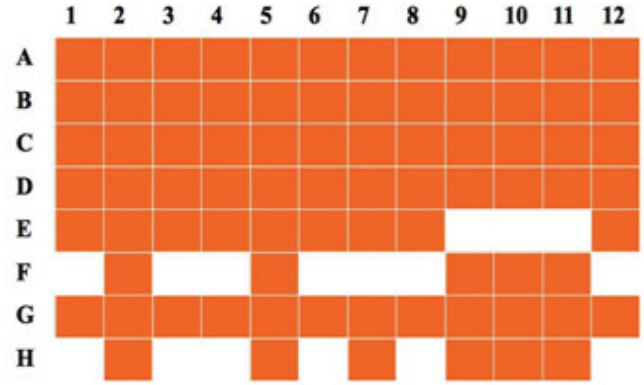

Figure 2

Photograph showing presence and absence of different types of phytochemicals in the bark from of the 12 samples; A) reducing sugars, B) tannins, C) phlobatannins, D) saponins, E) terpenoids, F) alkaloids, G) polyphenols and H) cardiac glycosides.

Antimicrobial activities

Plant extracts were screened against four different pathogens, the positive activity against pathogens were recorded as zone of inhibitions and data is shown in table 2 below

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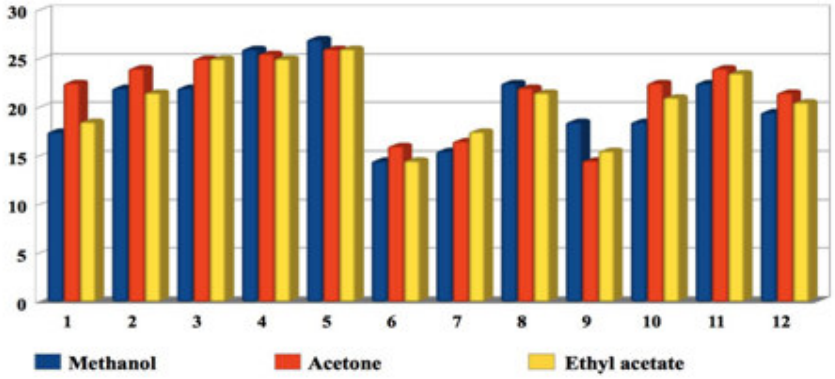

Figure 3
Data showing zones of inhibition of Acacia nilotica leaves extracts of all of the samples against P. aeruginosa.

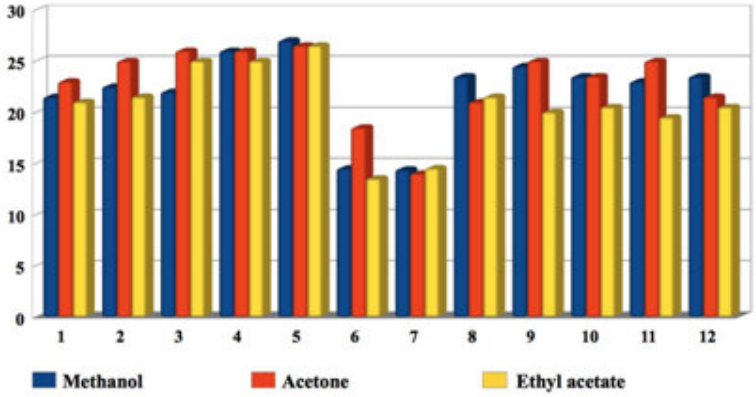

Figure 4
Data showing zones of inhibition of Acacia nilotica leaves extracts of all of the samples against S. aureus.

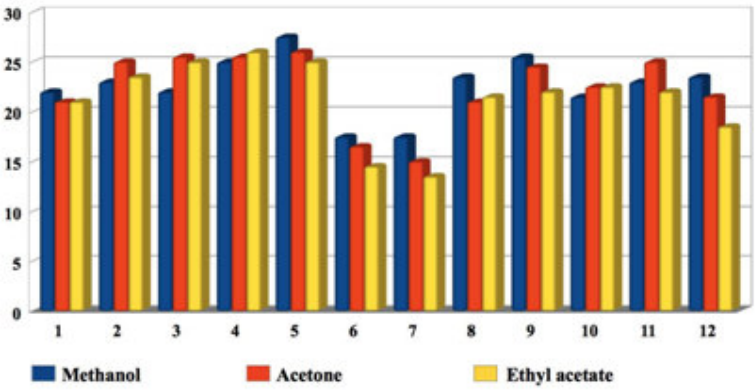

Figure 5
Data showing zones of inhibition of Acacia nilotica leaves extracts of all of the samples against E. coli.

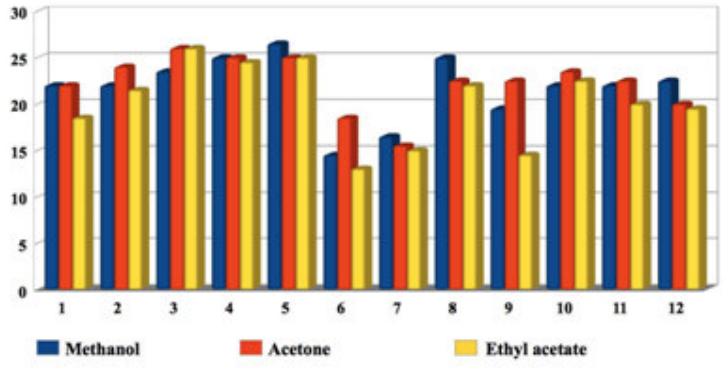

Figure 6
Data showing zones of inhibition of Acacia nilotica leaves extracts of all of the samples against B. subtilis.

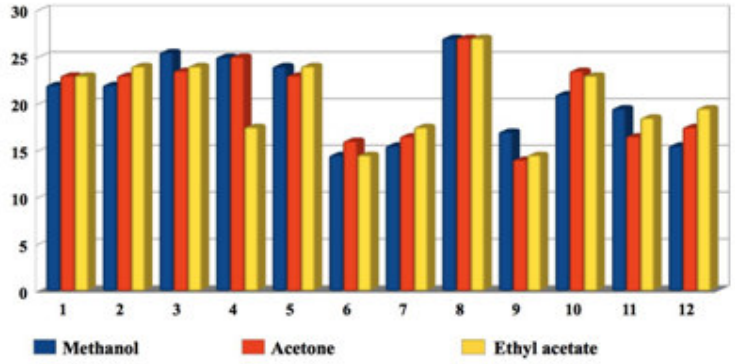

Figure 7
Data showing zones of inhibition of Acacia nilotica bark extracts of all of the samples against P. aeruginosa.

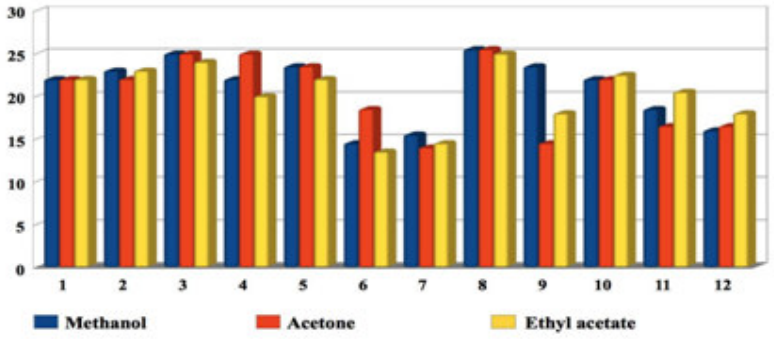

Figure 8
Data showing zones of inhibition of Acacia nilotica bark extracts of all of the samples against S. aureus.

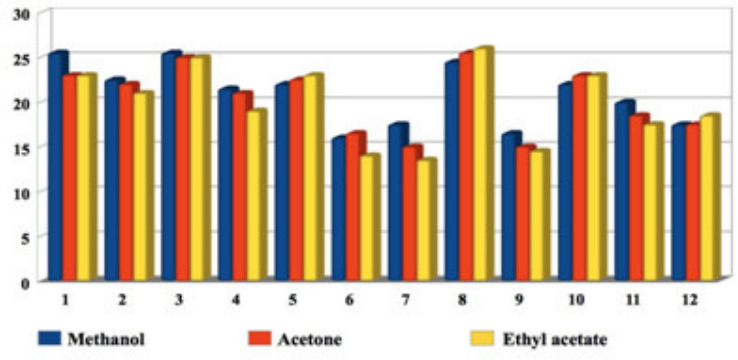

Figure 9
Data showing zones of inhibition of Acacia nilotica bark extracts of all of the samples against E. coli.

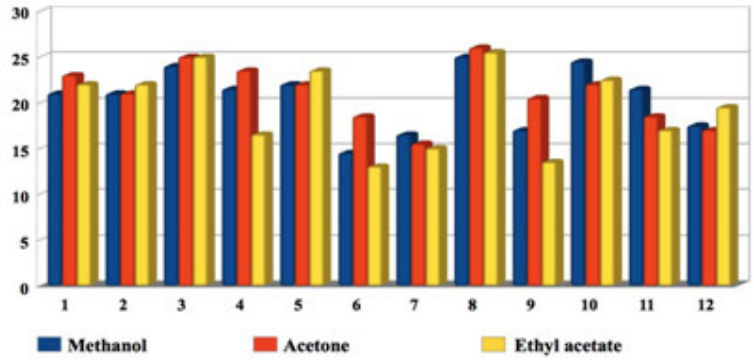

Figure 10
Data showing zones of inhibition of Acacia nilotica bark extracts of all of the samples against B. subtilis.

DISCUSSION

Plants used in Ayurveda can provide biologically active molecules and lead structures for development of modified derivatives with enhanced activity and/or reduced toxicity of the 2, 50, 000 higher plant species on the earth, around 5,000 species have specific therapeutic value (Irshad *et. al*, 2012). Our data suggests that the type of different phytochemicals varied in leaves and bark for almost all of the samples selected in the study (Fig. 1 and 2). A previous study has shown that the stem, roots and leaves of *A. nilotica* are commonly used in the traditional therapy of various diseases. At 10mg/ml concentration of plant extracts exhibited very good antibacterial activity against almost all of the four pathogens selected in our study (Fig. 3-10). The experiments were repeated in triplicates to verify the data and alterations and in each experiments, we analyzed a very good activity of *Acacia* against pathogens. Out of 12 samples selected in our study, only sample 6 and sample 7 were found comparatively less effective against pathogens as compared to remaining samples. Our preliminary data suggest that a effective approach towards identification and purification of the key compound is required to analyze its antimicrobial properties more closely for treatment of infectious diseases with herbal antibiotics of plant origin.

CONCLUSION

The current study has shown positive responses towards some reliable estimate of *Acacia nilotica against* four microbes; *P.aeruginosa, S. aureus,Ecoli and B.subtilis* among the examined bark and leaves extract with different solvents. In the current study,

almost extracts showed antibacterial activity against selected microbes in terms of zone of inhibition. It is also conferred that *Acacia nilotica* is rich in phytochemicals which warrants further in depth study to be a potent antimicrobial agents and phytochemical screening can serve as the basis for preparation of efficient herbal monograph for proper identification and authentication of drug for safer and more valuable therapeutic substitutes than synthetically created antimicrobial agents through phytochemical analysis.

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