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Phytochemical Estimation and Antimicrobial Activity of *Crotalaria paniculata* Extracts

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A B S T R A C T

The phytochemical constituents are very essential components responsible for various biological activities of medical importance. In this study, the ethanolic and water extracts of leaves and stem of *Crotalaria paniculata* were quantitatively analysed for various phytochemicals and also tested for their antimicrobial activity against *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichi coli*, *Pseudomonas aeriginosa*, *Pseudomonas cepacia*, *Shigella* sp., *Klebsiella pneumoniae*, *Salmonella typhi*, *Bacillus megaterium*, and *Bacillus subtilis*. Resazurin dye reduction method was used for antimicrobial assay while the phytochemicals viz., tannins and flavonoids were determined from plant extracts. The results also revealed that the alkaloides and phenols were higher in ethanol and water extract of stem compared to the leaf extracts.

Introduction

Secondary metabolites produced by plants constitute a major resource of bioactive substances. The scientific interest in these metabolites has improved today with the search of new remedial agents from plant source, due to the increasing development of the resistance pattern of microorganisms to presently used antimicrobial drugs (Mbosso *et al.*, 2010). Alkaloids of plants are essential bioactive substances, and their antimicrobial activity has been reported. Alkaloids reveal strong inhibitory activity in opposition to *Sclerotium hydrophilum*, *Penicillium italicum*, *Gaeumannomyces graminis* and *Rhizoctonia solani* (Zhu *et al.*, 1992). An antimicrobial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoa. Antimicrobial drugs either kill microbes or prevent the growth of microbes. Disinfectants are antimicrobial substances used on non-living matter or outside the body. Plants are major source of natural products, many of which can be used as pharmaceuticals, agrochemicals, flavour and fragrance ingredients, food additives, and pesticides (Chandran *et al.*, 2020). Several plant extracts such as *Andrographis* spp., *Hyptis suaveolens*,

Phyllanthus maderaspatensis, and others against Gram-positive and Gram-negative bacterial strains (Karmegam *et al.,* 2015, Karmegam *et al.,* 2008). Effects of phytochemical were conducted and it was observed the antimicrobial activity of anacardic acid on *Staphylococcus aureus, Brevibacterium ammoniagenes, Streptococcus mutans* and *Propionibacterium acnes*. Later, it was tested the bactericidal activity of anacardic acid and totarol on methicillin resistant strains of *Staphylococcus aureus* (MRSA) and the synergistic effect of these compounds associated with methicillin.

Eighteen plant species used in folklore medicine in west Nepal were tested for their antibacterial activity by the disk diffusion method. The bacteria employed were gram-positive (*Staphylococcus aureus)* and gram negative (*Escherichia coli, Pseudomonas aeruginosa* and *Shigella boydii*). Extracts of eight plants showed encouraging result against three strains of bacteria, while other showed activity against one or two strains.

These findings support the traditional knowledge of local users (Panthi and Chaudhary, 2006). As this is an interesting and useful piece of information, less confidence is placed on these values in determining the quantity of the drug known to the patients. The indigenous Jordanian plant extracts act against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa* by using XTT assay and viable count methods. *Hypericum triquetrifolium, Ballota undulata, Ruta chalepensis, Ononis natrix, Paronychia argentea* and *Marrubium vulgare* had shown promising antimicrobial activity by the both the methods. These studies illustrate that as both XTT and viable count methods are as good as when estimating the overall antimicrobial activity of experimental substances (Al-Bakri and Afifi, 2007). The selected plant *Crotalaria paniculata* is a specific medicinal plant contains many active components as well as many literature found used as traditionally against skin diseases. However, no literature found in component determination as well as antimicrobial properties, therefore the present study has been undertaken for quantitative phytochemical estimation and evaluate the antimicrobial potential of the ethanolic and water extract employing resazurin dye reduction besides analyzing the quantitative contents of phytochemicals.

Materials and Methods

The leaves and stem of *Crotalaria paniculata* (Fig.1) were collected and shade dried for a week and powdered using mortar and pestle. A fine powder obtained was stored in air tight poly bags and used for preparation of extract. Dried leaf and stem were powdered with using mortar and pastel. Dried powder was filled in Soxhlet apparatus and ethanol and water were used for preparation of extracts. The extracted plant material was stored in refrigeration condition for further use.

For ethanol extraction, the powdered plant material (100 g) was taken in a thimble and kept the thimble on round bottom flask. Added 250 ml of ethanol in that round bottom flask then fix the condenser on upper part of the thimble, then heated to 50 ^{o}C in heating mantle. These steps were carried for 24 hours until the extract in the siphon tube become colourless. When the extracts were settled in the boiling flask the heater had to be switch off the heater. When the flask gets heat again the same procedure was taken place on mention above. When the ethanol was separated in the thimble it was removed and discarded to recycled ethanol. After that the extracts were poured into a bowl kept it for drying purpose to get plant extract.

For water extraction air dried powdered samples (100 g) were used for the ethanol extraction and put it on the thimble and kept the thimble on round bottom flask. Then added 250ml of water in a round bottom flask, then the content was kept the condenser on upper part of the thimble. Then heat in a heating Mendel for boiling 60 ^{o}C for 24 hours. The water is flowed in to cool down the heat of vapour to change it again to liquid form. When the water changes into liquid it passed to the thimble drop by dropt to separate the extracts from the samples.

Fig.1 The plant used in the study, *Crotalaria paniculata*.

Antimicrobial activity assay

The selected bacterial strains are collected from NCIM (National centre for industrial microorganisms, Chandigarh). The following bacterial species are used for the study. *Micrococcus luteus*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichi coli*, *Pseudomonas aeriginosa*, *Pseudomonas cepacia*, *Shigella* sp., *Klebsiella pneumonia*, *Salmonella typhi*, *Bacillus megaterium* and *Bacillus subtilis*.

For culturing and maintain the bacterial species nutrient broth were prepared. Medium compositions were prepared and autoclaved at 15 lbs or 121°C for 15 minutes. Broth were transferred in to test tube culture was transferred in to culture tube. Then the tubes were incubated at 36°C and the bacterial growth was observed based on turbidity. Bacterial strains were cultured on Mueller-Hinton agar media with regular interval for subculture and stored in 20°±2°C. Stock cultures containing 1 x 10^7 cfu x ml (0.5 MacFarland) of each bacterial strains were saved frozen at -20°C, thawed when required to perform the test and grown for 2 days in complete nutrient agar broth.

The culture obtained were vortexed, large agglomerates allowed to sediment completely and the supernatant further diluted 1:5 in complete minimal broth Titres were determined by viable counting on haemocytometer under microscope,

giving 1 x 10^3 per ml. These strain dilutions were used as inoculum in both microtitre assay and colorimetric assay. Resazurin dye reduction method was employed for antimicrobial assay using the standard method (Karuppusamy and Rajasekaran, 2009). Minimum Inhibitory Concentration (MIC) was assessed based on the results obtained.

Quantitative analysis of phytochemicals

The phytochemicals such as phenol compounds, tannins and flavonoids were determined from plant extracts as described by Makkar and co-workers with slight modification (Makkar, 2000). The level of ascorbic acid in the sample was quantified spectrophotometrically by the method described by Roe and Kuether (1943).

Results and Discussion

The resazurin reduction test can be used for colorimetric determination of MIC of the plant extracts on par with earlier method. After 5 hours of inoculation of sample extracts in different concentrations with marker dye solution were taken the absorbancy of the cultured broth. The colour changes in the tubes can be markedly visible and also obtained MIC (maximum absorbancy) for potential antibacterial extracts showed the values close to the antibiotic control wells. The MIC values of *Micrococcus leuteus* showed the range of concentration 62.5 µl which is more or less equal to reference antibiotic concentrations. These two parts of the medicinal plant extract inferred that they are having more potential antibiotic properties against selected bacteria. The moderate bacterial susceptibility and MIC was obtained from the plant extracts of *Bacillus subtilis*. Remaining plant extracts showed that minimum susceptibility against the selected bacterial strains which all extracts possess the antibacterial property above 250 µL concentrations.

From the above result shows the control is having 62.5 µL it contain MIC but the selected medicinal plant extracts (Leaf water, stem ethanol and stem water) is possesses very high antibacterial activity i.e, the MIC is 32.5 µL when compare with control the MIC value is very low. However, leaf ethanol is also containing 62.5 µL MIC value. The control is also containing 62.5 µL. From this result highest antibacterial activity is present in the selected medicinal plant.

Fig.2 Quantitative contents of phytochemicals in leaf extracts of *Crotalaria paniculata*. Values are mean of triplicate values expressed in µg/ml.

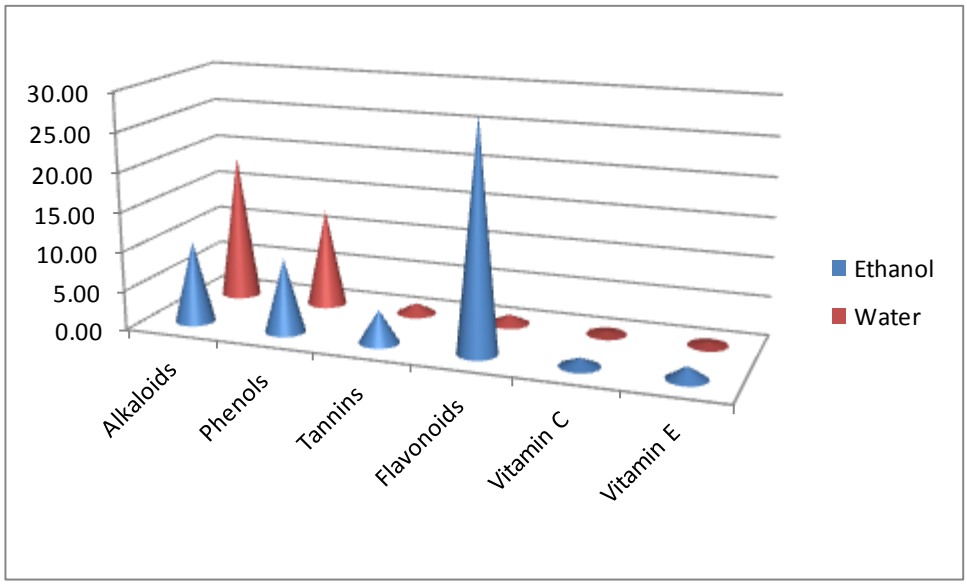
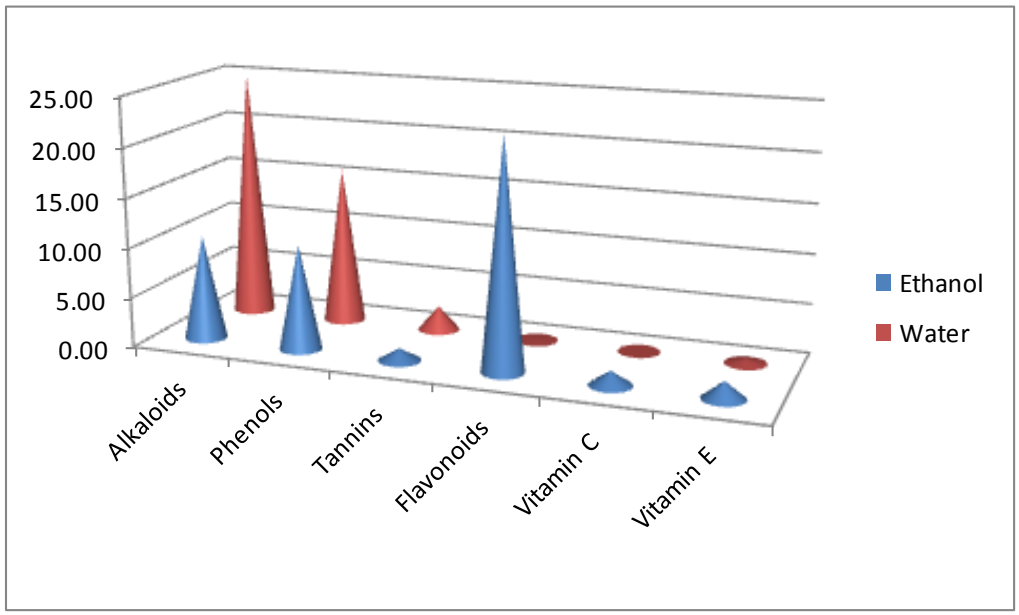


Fig.3 Quantitative contents of phytochemicals in stem extracts of *Crotalaria paniculata*. Values are mean of triplicate values expressed in µg/ml.



The selected medicinal plant *Crotalaria paniculata* leaf and stem were taken for the study. The selected plant extracts showed maximum antibacterial activity is obtained. This is the first attempt to screening the antibacterial activity resazurin reduction method.

The result revealed that the selected medicinal plant extracts is act against *K. pneumoniae*, *S. aurues* and *S. typhi* strains. This is containing very high antibiotic sources. The further study is purifying the plant extracts and isolates the compound and verifies the antibacterial activity. *B. subtilis*, *S. aeruginosa* and *M. luteus* are moderately controlled by selected plant extract. It might be the purified compounds are able to control the organisms. *E. coli*, *P. epidermis*, *B. megaterium* and *P. cepacia* strains are very low activity against medicinal plant extract. However, the selected medicinal plant extract is act against three bacterial strains. The MIC values against the bacterial species are highly pathogen to human beings. The selected medicinal plant extract is recommended to prepare the drug and it will be use for preparation of new natural therapeutic agent. The flavonoids content in ethanolic extract was higher (28.98 ug/ml) than that of the water extract of Crotalaria paniculata leaf; while it was 22.93 ug/ml in the ethanolic stem extracts (Fig. 2 and Fig. 3). The results also revealed that the alkaloides and phenols were higher in ethanol and water extract of stem compared to the leaf extracts. This shows that the phytochemicals vary with references to the type of solven used.

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