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Antibacterial activity of Alcoholic and Aqueous extracts of some Medicinal Plants

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Abstract: The alcoholic and aqueous extracts of fourteen medicinal plants collected from Uttarakhand, North India; were evaluated for antibacterial activity by Agar diffusion method against medically important bacteria viz. *B. subtilis, K. pneumonia, E. coli, Enterobacter aerogenes, Pseudomonas aeruginosa, S. auresus* and *P. mirabilis* .Kanamycin was used as standard drug for antibacterial activity. Out of fourteen plant extracts, alcoholic extract of *Curcuma longa* showed the best antibacterial activity.
Key words: medicinal plants, antibacterial activity, alcoholic extracts , aqueous extracts.

Introduction

Medicinal plants are gifts of nature to cure limitless number of diseases among human beings .It has been well known since ancient time that plants and spices have antimicrobial activity. There has been a considerable interest to use plants and spices for the elimination of microorganisms because of increasing antibiotic resistance of microorganisms.
According to World Health Organization (WHO) more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used in traditional medicine contain a wide range of ingredients that can be used to treat chronic as well as infectious diseases. A vast knowledge of how to use the plants against different illnesses may be expected to have accumulated in areas where the use of plants is still of great importance [1].

Now a days multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases[2,3]. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reactions[4]. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance[5], there is a constant need for new and effective therapeutic agents[6]. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants[7,8]. Several screening studies have been carried out in different parts of the world. There are several reports on the antimicrobial activity of different herbal extracts in different regions of the worlds[9,10].

Consideration to all the mentioned facts, the present study has been focused to extract different medicinal plants each belonging to different families for their antibacterial properties.

Materials and Methods

Collection of Samples

• The plant samples were collected from North India mainly from Uttarakhand (India). They were kindly identified by professor Dr.Ajay Swami (Taxonomist) of Botany Department, C.D.College, Haridwar (Uttarakhand) .

• Fresh plant materials were washed separately under running tap water, air dried, and then homogenized to very fine powder and stored in air-tight container protected from direct sunlight, refrigerated until required for use [11].

Crude Extraction [12]

• Hot Water Extract (HWE) of the respective plant samples were made by dissolving 150g, of the powdered plant sample in 200ml. of distilled water for 4 hours. It was then further extracted using the soxhlet apparatus for a further 2hrs. The resulting infusion was filtered using Whatman no.1 filter paper. The filtrate was then subjected to gentle evaporation using a hot plate. The resulting paste was then scrapped onto a watch glass where it was allowed to evaporate to dryness in an oven at 60°C. The HWE was then ground, weighted and stored in the powdered form in an airtight container in a refrigerator until required.

• The Alcohol Extract was made by soaking 100g of each powdered plant material in a solution of 300-400 ml. of 95% ethanol or methanol. The mixture was allowed to stay for 48-72 hrs in dark away from direct sun-light. It was stirred at 12 hr. intervals by means of sterile glass rod. The resulting liquid was filtered using Whatman filter paper no.1 .This process of extraction was repeated with the same volume of alcohol(ethanol/methanol).The filterate was evaporated gently to dryness and weighed. It was stored in the same condition as HWE.

Qualitative Anti-bacterial Studies

Materials and method used

Method followed: - Agar Diffusion Method

Requirements: - Petridishes, glass syringes, cork borers (all sterilized by dry heat)

Working procedure:-

Preparation of test and standard solutions:-The test solutions of the extracts were prepared in distilled DMSO at a concentration of 1, 5 and 20 mg / ml. Kanamycin was used as standard and was dissolved in distilled DMSO to get a final concentration of 30µg / ml. DMSO (0.1 ml) was used as solvent control.

Microorganisms used:-

The *Bacillus subtilis* (NCIM 2439), *Klebseilla pneumonia* (NCIM 2065), *Escherichia coli* (NCIM 2345), *Enterobacter aerogenes* (NCIM 2340), *Pseudomonas aeruginosa* (NCIM 2200), *Staphylococcus auresus (*NCIM 2200) and *Proteus mirabilis* (NCIM 2241) strains were employed for the present study. The microorganisms were maintained by sub-culturing and used at regular intervals in nutrient agar medium.

Preparation of Inoculums [13]:-

The suspensions of all the organisms were prepared as per Mac-Farland Nephelometer Standard. A 24 hour old culture was used for the preparation of bacterial suspension. Suspensions of organisms were made in sterile isotonic solution of sodium chloride (0.9% w/v) and the turbidity was adjusted.

Preparation of assay media [14]:-

Culture medium

The following media were used for the antimicrobial studies.

- Nutrient broth

Beef extract	0.15%
Sodium chloride	0.5%
Peptone	0.5%
Yeast extract	0.15 %

37 g of above readymade powder was dissolved in 1 L of distilled water; pH was adjusted to 7.8 and sterilized by autoclaving at 15 lbs for 15 min.

- Nutrient Agar

Beef extract	1.00%
Sodium chloride	0.5%
Peptone	1.0%
Agar	2.5 %
pH	7.0-7.2

The sterilized medium was cooled to 40º C and poured into petridishes to obtain 4-6 mm thickness. The media was allowed to solidify at room temperature.

Sterilization:-

Sterilization of media, peptone water, distilled water etc., was carried out by autoclaving at 15 lbs for 20 min. The glassware was sterilized by dry heat in an oven at a temperature of 160 ºC for one hour [14].

Procedure:-

A sterile borer was used to prepare cups of 10 mm diameter in the agar media spread with the

microorganisms. 0.1 ml of inoculums (of 10^4 to 10^6 CFU / ml population prepared from standardized culture, adjusted with peptone water) was spread on the agar plate by spread plate technique. Accurately measured (0.1 ml) solution of each extract and standard samples were added to the cups with a micropipette. All the plates were kept in a refrigerator at 2 to 8 ºC for a period of two hours for effective diffusion of test compounds and standards. Later, they were incubated at 37 ºC for 24 h. The presence of definite zones of inhibition around the cup indicated antibacterial activity. The solvent control was run simultaneously to assess the activity of DMSO, which was used as a solvent for extracts. The diameter of the zone of inhibition was measured and recorded [15].

Result: Table 1: Antibacterial activity (zone of inhibition in mm.)

Plant Extracts	*B.subtilis*	*K.pneumonia*	*E.coli*	*Enterobacter aerogenes*	*Pseudomonas aeruginosa*	*S.auresus*	*P.mirabilis*
Cuscuta refiexa							
Aqueous	------	------	------	------	-----	------	04
Alcoholic	04	-----	06	-----	-----	06	08
Achyranthes aspera							
Aqueous	06	------	------	------	-----	08	-----
Alcoholic	04	-----	-----	-----	-----	10	10
Alangium salvifolium							
Aqueous	-----	------	------	------	-----	------	------
Alcoholic	06	-----	04	-----	-----	08	------
Calotropis procera							
Aqueous	------	------	------	------	------	06	------
Alcoholic	-----	-----	------	-----	04	08	------
Clerodendrum serratum							
Aqueous	05	06	------	------	------	-----	------
Alcoholic	12	-----	08	-----	04	04	05
Phyllanthus fraternus							
Aqueous	------	-----	------	------	------	-----	------
Alcoholic	06	-----	-----	-----	-----	08	-----
Adhatoda zeylanica							
Aqueous	04	-----	------	------	------	-----	------
Alcoholic	08	05	-----	04	-----	-----	-----
Euphorbia thymifolia							
Aqueous	-----	------	------	------	------	------	-----
Alcoholic	10	-----	-----	-----	-----	06	06
Cassia tora							
Aqueous	05	------	------	------	------	------	05
Alcoholic	08	-----	06	-----	08	05	-----
Valeriana wallichii							
Aqueous	08	------	-----	-----	------	-----	06
Alcoholic	12	04	-----	04	-----	05	08
Solanum nigrum							
Aqueous	-----	------	------	------	05	------	------
Alcoholic	-----	-----	06	------	10	-----	------
Asparagus racemosus							
Aqueous	-----	------	------	------	------	------	------
Alcoholic	05	-----	04	------	------	06	04
Curcuma longa							
Aqueous	12	------	10	06	------	08	10
Alcoholic	18	14	17	15	14	16	18
Woodfordia fruticosa							
Aqueous	-----	------	-----	------	------	-----	-----
Alcoholic	-----	08	04	------	-----	06	-----
Kanamycin (30µg/disc)	23	20	22	21	20	24	21
Control (DMSO)	----	----	----	----	----	----	----

---- = No Activity

Discussion

The presence of antimicrobial substances in the higher plants is well established . Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health .

Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines or it can be the base for the development of a medicine ,a natural blueprint for the development of a drug .

Successive extraction and isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent but we found in this study that the plant extracts by alcohol(ethanol) provided more consistent antibacterial activity compared to those extracted by water .

The results of antibacterial activity of all the medicinal plants against the investigated bacterial strains are shown in Table 1.

The higher antimicrobial activity of most of the alcoholic extracts as compare to aqueous extracts might be due to the lack of solubility of active constituents in aqueous solution.

Out of fourteen plant extracts tested for their antibacterial activity, *Curcuma longa* showed most promising activity.

References

1. Diallo D., Hveem B., Mahmoud M.A., Betge G., Paulsen B.S. and Maiga A., An ethnobotanical survey of herbal drugs of Gourma district, Mali. Pharmaceutical Biol.,1999, 37:80–91.
2. Davis J., Inactivation of the antibiotics and the dissemination of resistance genes, Science, 1994,264,375-382 .
3. Service R.F., Antibiotics that resist resistance, Science,1995,270,724-727 .
4. Ahamad I., Mehmood Z. and Mohammad F., Screening of some Indian medicinal plants for their antimicrobial properties, J. Ethno pharmacol,1998,62,183-193.
5. Monroe S. and Polk R., Antimicrobial use and bacterial resistance, Curr.Opin.Microbial, 2000 ,3,496-501 .
6. Bhavnani S.M. and Ballow C.H., New agents for Gram-positive bacteria, Curr. Opin. Microbial ,2000 ,3,528-534 .
7. Clark A.M., Natural products as resource for new drugs, Phar. Res., 1996 ,13,1133-1141.
8. Cordell G.A., Biodiversity and drug discovery a symbiotic relationship, Phytochemistry, 2000, 55,463-480 .
9. Nair R. and Chanda S.V., Antibacterial activity of some medicinal plants of Saurashtra region, J. Tissue Res., 2004,4,117-120 .
10. Nair R., Kalariya T. and Chanda S., Antibacterial activity of some selected Indian medicinal flora, Turk. J. Bio., 2005,29 ,41-47 .
11. Parekh J. and Chanda S., *In vitro* screening of antimicrobial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae, African J. Micro.Res., 2007, 1(6), 92-99.
12. Nwachukwu I.N., Allison L.N.-O., Chinakwe E.C. and Nwadiaro P.,Studies on the effects of *Cymbopogon citrates*, *Ceiba pentandra* and *Loranthus bengwelensis* extracts on species of dermatophytes., The J. American Sci., 2008, 4(4), 52-63.
13. Cremer A., Microbiological methods, Butter worth and Co., London, 6th Ed, 1991,235.
14. R Cruikshank J.P., Duguid B.P., Marmion R.H.H. and Smain, Medical Microbiology, Vol-2, Churchill Livingstone, New York, 1995, 190.
15. Ali M.S., Ahmad V.U., Usmanghani K., Azhar I. and Amtul Z., Antimicrobial screening of some *Caesalpiniaceae*, Fitotherapia., 1999, 70, 299-304.

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