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Evaluation of Antimicrobial Activity of Alcoholic and Aqueous Extracts of Five Plants used in Traditional Medicine in North India

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Abstract: The alcoholic and aqueous extracts of five medicinal plants (*Cassia fistula, Albizia lebbeck, Cassia occidentalis , Sphaeranthus indicus* and *Vitex nigundo*) 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, P. mirabilis* .The alcoholic extracts showed some degree of antibacterial activity as compare to aqueous extracts. Kanamycin was used as standard drug for antibacterial activity. Out of five plant extracts, alcoholic extract of *Sphaeranthus indicus* showed the best antibacterial activity.
Key words: medicinal plants, antibacterial activity, alcoholic extracts , aqueous extracts.

INTRODUCTION

Traditional health practices are based on many years of experience acquired by humans in their journey in evolution. Primitive man sought remedies for illnesses from his surrounding animal, vegetable and mineral kingdoms. Some of these remedies are based on mistaken beliefs or mysticism. However, as civilization progressed, particularly in Asia and notably in China, Egypt, India and the Middle East, the rich treasure of knowledge on herbal remedies was systemically preserved, collated and written about, based on the prevailing concepts of health and disease. This resulted in the development of different systems of medicine like the Chinese system, Ayurveda, Unani and Siddha. Ebers Papyrus of ancient Egyptians or the Samhitas of Ayurveda, were veritable treasure houses of human knowledge on herbal remedies and ethno-therapeutics.

Herbal medicines are complex mixtures of plants, animal parts or products, mineral and metals. However, plants and plant products form the dominant part of Materia Medica of traditional medicine practiced in different parts of the world and in particular in Asia, especially China, India, Korea, Philippines, Indonesia and Tibet. In India, for example, the Charaka Samhita (treatise), dating back to 900 BC, lists 341 plants and plant products for medicinal use. Susruta, who practiced surgery about 600 BC, described 395 medicinal plants[1].

Medicinal plants are a source of great economic value in the Indian subcontinent. Nature has been bestowed on us a very rich botanical wealth and a large number of diverse type of plants grow in different parts of the country.

India is rich in all the 3 levels of biodiversity, namely species diversity, genetic diversity and habitat diversity. In India thousands of species are known to have medicinal value and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times.

Herbal medicine is still mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and fewer side effects. However, the last few years have seen a major increase in their use in the developed world.

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].

Side effects and the resistance that pathogenic microorganisms build against antibiotics, recently much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine[11]. Plant-based antimicrobials represent a vast untapped source of medicines and further exploration of plant antimicrobials need to occur. Antimicrobials of plants origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials.

All plants containing active compounds are important. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. These compounds are more complex and specific and are found in certain taxa such as family, genus and species, but heterogeneity of secondary compounds is found in wild species[12]. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct[13]. The plant's secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites. Therefore, random screening of plants for active chemicals is as important as the screening of ethnobotanically targeted species.

The need of the hour is to screen a number of medicinal plants for promising biological activity.

Consideration to all the mention facts, the present study has been designed to extract different medicinal plants each belonging to different families for their antimicrobial properties.

MATERIALS AND METHODS

Collection of Samples

- The plant samples were collected from North India mainly from Uttarakhand (Haridwar, Roorkee, Dehradun etc. places), 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 [14].

Crude Extraction [15]

- 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 # 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 48-72hrs in dark away from direct sun-light. It was stirred at 12hr. 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 [16]:-

The suspensions of all the organisms were prepared as per Mac-Farland Nephelometer Standard . A 24 h 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 [17]:-

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 [17].

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 [18].

RESULT AND DISCUSSION

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

Plant Extracts	B.subtilis	K.pneumonia	E.coli	Enterobacter aerogenes	Pseudomonas aeruginosa	S.auresus	P.mirabilis
Cassia fistula							
Aqueous	------	------	------	------	04	------	------
Alcoholic	10	08	------	-----	06	11	------
Albizia lebbeck							
Aqueous	04	------	------	------	------	------	------
Alcoholic	14	08	------	------	------	12	07
Cassia occidentalis							
Aqueous	------	------	------	------	------	03	------
Alcoholic	08	------	------	03	03	05	05
Sphaeranthus indicus							
Aqueous	07	------	------	------	------	04	04
Alcoholic	11	10	05	04	06	15	------
Vitex nigundo							
Aqueous	------	------	------	------	------	------	------
Alcoholic	04	05	------	-----	04	04	04
Kanamycin(30µg/disc)	23	20	22	21	20	24	21
Control (DMSO	----	----	----	----	----	----	----

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 antimicrobial activity compared to those extracted by water .

The results of antibacterial activity of five 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 five plant extracts tested for their antimicrobial activity *Spheranthus indicus* showed most promising activity and their alcoholic extracts are more effective as compare to aqueous extracts. But this plant extract is less active than standard kanamycin.

Most of the plant extracts(alcoholic and aqueous) showed less activity in case of Gram negative bacteria while the Gram positive *B.sutilis* was the most susceptible bacteria followed by *S.aureus.*

Various workers have already shown that Gram positive bacteria are more susceptible towards plant extracts as compared to Gram negative bacteria .These differences may be attributed to fact that the wall in Gram positive bacteria is of single layer , whereas the Gram negative cell wall is multilayered structure .

Amongst the plant species investigated , alcoholic extracts of *Spheranthus indicus* showed the most remarkable activity against all the microorganisms .

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