Study of antibacterial effect of some selected medicinal plant extracts

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Abstract: Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Plants have become the base for the development of a medicine, a natural blueprint for the development of new drugs and phytomedicine now a days are used for the treatment of many disease. The aim of this study was to evaluate the antimicrobial activity of medicinal plants used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms. Therefore, extracts of the ten plants i.e. *Ocimum Sanctum* (Tulsi), *Jatropha curcas, Azarichta indica* (Neem), *Eucalyptus* (Nilgiri), *Hibiscus rosasinesis* (Shoe flower), *Emblica phyllanthus* (Amla), *Carica papaya* (Papaya), *Syzygium cumini* (Jambhul), *Acasia nilotica* (Babul), *Ficus religiosa* (Peepal) were evaluated for their antibacterial potentials. The ethanolic and methanolic extracts of Emblica phyllanthus, Eucalyptus, Acacia nilotica and Syzgium cumini were the most efficient in inhibiting almost all the tested organisms.

Keywords: Antibacterial, plant Extracts, phytomedicine, Agar diffusion

I. INTRODUCTION:

During the last century, the practice of herbalism became the main stream throughout the world. In spite of great advances observed in modern medicine, plants still make an important contribution to health care. This is due to the recognition of the value of traditional medical systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopoeias, which have significant healing power. (Kubmarawa et al., 2007). Medicinal plants have been used for centuries before the advent of orthodox medicine. Leaves, flowers, stems, roots, seeds, fruit, and bark can all be constituents of herbal medicines. The medicinal values of these plants lie in their component phytochemicals, which produce definite physiological actions on the human body. The most important of these phytochemicals are alkaloids, tannins, flavonoids and phenolic compounds (Afolabi et al., 2007). Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999).

The problem of microbial resistance to antibiotics is growing and the future of antimicrobial drugs is still uncertain. Therefore, appropriate actions have to be taken to reduce this problem of emerging drug

resistance. To control drug resistance among microorganisms, alternative source of antibiotic should be looked out for. According to the world health organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Boyd *et al.,* 1994). Therefore, such plants should be investigated to better understand their antimicrobial property. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection fighting strategies to control microbial infections. (Sieradzki *et al*., 1999).

In the present study 10 medicinal plants were selected to evaluate their effect of antibacterial properties.

II. MATERIALS AND METHODS:

Sample collection /identification:

The leaves of the 10 medicinally important plant species (*Ocimum Sanctum* (Tulsi), *Jatropha curcas, Azarichta indica* (Neem), *Eucalyptus.sp* (Nilgiri), *Hibiscus rosasinesis* (Shoe flower), *Emblica phyllanthus* (Amla), *Carica papaya* (Papaya), *Syzygium cumini* (Jambhul), *Acasia nilotica* (Babul), *Ficus religiosa* (Peepal)) were collected from Agriculture research center, Badnapur. The leaves of the plant species were identified by standard monographs. The leaves were thoroughly washed with distilled water and kept for air-drying under shade. The leaves of the plant species were dried to a constant weight. After air drying the leaves are then finely powdered by using a blender and were then used for extraction.

Preparation of Plant Extracts:

The stock solutions of the extracts were prepared by taking the fine powder of the leaves and dissolving in the solvents. The powder was weighed and added to 10 ml of solvent i.e. distilled water, ethanol and methanol respectively. These stocks were kept on rotary shaker for 24 hours at normal room temperature for extraction. After 24 hours the stock solutions were filtered through the muslin cloth and then centrifuged at 5000 rpm for 15 min. After centrifugation the supernatant was collected and stored at 4ºC in tubes until further use.

Test Organisms:

The bacterial cultures were obtained from nutrient agar slants and then activated by suspending bacterial isolates from slants into 5 ml nutrient broth and then incubated for 24 hours at 37ºC.

5 different bacterial species were used for the study namely *Staphylococcus aureus , Proteus mirabilis, Escherichia coli, Bacillus megaterium, Bacillus subtilis, Shigella*.

Antibacterial activity:

The agar well diffusion method (Lino and Deogracious, 2006) was used in the present study. Standardized inoculum of each test bacterium i.e. 10 ul was spread onto sterile nutrient agar plates so as to achieve even growth. The plates were allowed to dry and a sterile cork borer was used to bore wells in the agar plates. 20 ul of each plant extract was added to the well against each bacterial species. The plates were kept for pre-diffusion and then incubated at 37°C for 24 hours. After incubation the zone of inhibition from each plates was noted. Standard antibiotic disc were also placed in prefilled bacterial culture plates of the test bacteria for comparison of zone of inhibition.

III. RESULTS AND DISCUSSION:

All the extracts of the plant tested showed varying degree of antimicrobial activities against the test bacterial (Table 1).

Table 1. Antibacterial activity of the 10 plant extracts in different solvents against pathogenic bacterial species

Extract	Solvents	*E.col*	*B.Subtilis*	*B.Megaterium*	*P.vulgaris*	*Shigella.sp*	*S.aureus*
		Mean diameter of zone of inhibition (cm)					
Ocimum sanctum	Aq	R	R	R	R	R	R
	Eth	0.66	R	R	R	0.92	0.52
	Met	0.72	0.72	R	R	0.4	0.26
Azadirachta indica	Aq	R	R	R	0.52	R	R
	Eth	1.06	1.12	0.32	0.8	0.6	0.4
	Met	1.26	0.4	0.4	0.46	0.6	0.46
Eucalyptus sp	Aq	R	3.72	1.2	R	0.4	R
	Eth	0.72	1.2	1.32	1.2	1.12	1.26
	Met	1.4	0.72	0.6	0.8	0.4	1
Jatropha curacus	Aq	R	R	R	R	R	R
	Eth	0.6	R	R	R	R	R
	Met	1.12	R	R	R	R	R
Hibiscus rosasinesis	Aq	R	R	R	R	R	R
	Eth	R	0.26	0.46	R	R	R
	Met	R	R	R	R	R	R
Caricapapaya	Aq	R	R	R	R	R	R
	Eth	0.86	R	R	R	0.32	R
	Met	1.06	R	R	R	R	R
Emblica phyllanthus	Aq	R	1.52	R	R	0.92	1.32
	Eth	1	1.06	1.8	1.4	1.2	1.4
	Met	2	0.8	1.4	1.6	0.86	1.52
Acacia Nilotica	Aq	R	1.86	R	R	R	R
	Eth	0.92	0.6	0.52	0.38	0.4	1.2
	Met	1.6	1.12	0.8	0.8	1.52	1.26
	Aq	R	R	R	R	R	R

Ficus Religiosa	Eth	R	R	R	R	R	R
	Met	R	R	R	R	R	R
Syzgium Cumini	Aq	R	R	R	1.12	0.66	0.66
	Eth	1.12	1	0.92	1.06	0.92	0.92
	Met	1.8	1.12	0.8	1.12	0.46	0.66

R = resistance. Aq = aqueous, Eth = ethanol, Met = methanol, Sol = solvents

The antimicrobial activities of the ethanol and methanol extracts compared favorably with that of the three standard antibiotics streptomycin, penicillin and tetracycline (Table 2) and have appeared to be broad spectrum as its activities were independent of gram reaction.

Table 2. Zone diameter recorded of the standard antibiotics against pathogenic bacteria.

Standard Antibiotics	*E.coli*	*B.Subtilis*	*B.Megaterium*	*P.vulgaris*	*Shigella.sp*	*S.aureus*
Penicillin	0.6	2.8	R	0.4	0.8	1.0
Streptomycin	1.4	1.4	R	1.6	2.0	2.0
Tetracycline	1.4	1.4	1	1.2	1.6	1.8

Maximum of the plant species showed effect against the test organisms, while some of bacterial species were resistant against the extracts. The effect that were seen was efficient against both gram positive and gram-negative bacteria. Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditional healers used primarily water as the solvent but studies suggest that plant extracts in organic solvent (methanol) provided more consistent antimicrobial activity compared to those extracted in water. These are in terms of the polarity of the compounds being extracted by each solvent. (Jigna Parekh et al., 2005)

Fresh or dried plant material can be used. However, most scientists working on the chemistry of secondary plant components have tend to use dried plant material. Differences in water content may affect solubility of subsequent separation by liquid-liquid extraction and the secondary metabolic plant components should be relatively stable, especially if it is to be used as an antimicrobial agent. (Dilika et al, 1996; Baris et al., 2006).

Many of the tested bacteria were resistant against aqueous extract of the plant species. The ethanolic and methanolic extract of the plant species showed significant result compared to aqueous extract. Methanol proved to be the most efficient solvent for extraction of bioactive compounds from the test plant species.

The highest zone of inhibition was shown by Methanolic extract of *Embilca phyllanthus* (mean diameter 2 cm) against *E.Coli,* followed by Methanolic extract of *Syzgium Cumini* (mean diameter 1.8 cm) against *E.ColiI* and methanolic extract of *Acacia Nilotica* (mean diameter 1.52 cm) against

Shegella sp.

The antimicrobial activity of the plant material is linked to the secondary metabolites present in it. Drugs contained in medicinal plants are called active principles and these active principles are divided into a number of groups. Carbohydrates present in plants are mostly in the form of pentoses, sucrose and soluble sugars. There is presence of glycoside moieties like saponins, anthracene and cardiac glycolsides, Some of which are known to structurally resemble sex hormones (oestrogens, gestrogens and androgens) are known to protect against gastric infections caused by enteric pathogens (Salmonella paratyphi) thus justifying the use of this plant in traditional medicinal practice (El-Mahmood et al., 2008).

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