Analysis of Antimicrobial Properties of some Ethnomedicinal Plants

Abstract

The world is suffering through large number of diseases which are primarily caused by the microbes, though it is bacteria, fungi, viruses or protozoans. The inappropriate, prolonged use of antibiotics and loss of biodiversity and climate change is leading to the invasion of microbes in the human population, because of which every individual on the earth is suffering from diseases caused by microorganisms. In current scenario it is the prerequisite to screen the effective, safe, cheap, and available therapeutics from plants and natural products. Plant parts of sixteen medicinal plant species such as *Aegle marmelos, Allium cepa, Allium sativum, Azadirachta indica, Calotropis procera, Cassia fistula, Catharanths roseus, Coriandrum sativum, Curcuma longa, Emblica officinalis, Eucalyptus, Mentha piperita, Nerium oleander, Ocimum sanctum, Withania somnifera* and *Zingiber officinale* with high antimicrobial activity against Gram-positive bacteria *Bacillus subtilis*, Gram-negative *Escherichia coli* and fungus *Aspergillus niger* were extracted with aqueous, ethanol and acetone and with the help of well diffusion method minimal inhibitory concentration (MIC) values were determined.

Out of three solvent phases, ethanol extract showed the highest inhibition against the microbes, where as the efficacy of most of the aqueous and acetone plant extracts were also confirmed as antimicrobial agent and their use as therapeutic drugs for the treatment of various diseases.

Keywords

Plant extracts, antimicrobial agents, zone of inhibition and medicinal plants

Introduction

Medicinal herbology is a trend of modern life culture in global perspective. In current scenario everyone is facing health changes because of microbial agents. Through the knowledge people can treat themselves, against various alignments. Since, ancient times India is known as repository of various herbs used for medical practices. About 80% people round the globe has been now shifted towards the herbal medicines for their primary care of ailments. Treatment through the natural products or herbs are always considered safe and without side effects. A vast number of natural products are known as antimicrobial agents which helps to treat the infectious disease [1]. Secondary metabolites of the plants make them a potential source of antimicrobial agents [2,3]. Alkaloids, flavanoids, phenols, saponins and tannins are the most common compounds found in the natural herbs and compounds [4].

Now a days new microbes have been raised which shows high resistance towards the new antimicrobial agents, this leads to explore the new potential agents against those harmful microbes [5, 6].

In view of vast application of natural herbs as antimicrobial agent, the main focus of this this

study is to investigate *in vitro* antibacterial and antifungal activity of extracts from some selected medicinal plants and to find out the effective solvent phase of plant extracts.

2. Materials & Methods

2.1 Sample Collection

To analyze the antimicrobial properties of the plants, 16 plant materials were collected from the local market. **Table 1 shows the botanical name, common name, family, parts used, active compounds present and the medicinal uses of the plants under study.**

BOTANICAL NAME	COMMON NAME	FAMILY	PART USED	ACTIVE COMPOUND	MEDICINAL USES
Aegle marmelos	Stone Apple or Bael	Rutaceae	Leaves	Saponins, Terpenoids Phenols	Diarrhea, dysentery
Allium cepa	Onion or Pyaz	Liliaceae	Bulb	Flavanol, Sulphur	Diuretic, expectorant, anti-tumor, cough, jaundice, splenic enlargement, dyspepsia, colic and scurvy.
Allium sativum	Garlic or Lehsun	Liliaceae	Bulb	Allicin	Fevers, cough, flatulence, disorders of nervous system, pulmonary phthisis, whooping cough, earache, anthelmintic
Azadirachta indica	Neem	Meliaceae	Leaves	Azadirachta, Margesic Acid	Jaundice, skin diseases, discutient, antiseptic, boils, chronic ulcers, small pox, glandular.
Calotropis procera	Rubber Bush or Mudar	Asclepiadaceae	Leaves	Cardenolides, Steroids, Tannins	Dropsy, asthma, cough, Skin diseases, leprosy, taenia, rheumatism, cold,
Cassia fistula	Golden Shower or Amaltas	caesalpiniaceae	Leaves	Flavonoids, β- sitosterol, β-D glucoside	Diabetes, ringworm, chilblains. purgative.
Catharanths roseus	Periwinkle or Sadabhar	Apocynaceae	Leaves	Vinblastine, Vindoline, Vincristines Alkaloids	Diabetes, wasp stings, menorrhagia, antibacterial used as stomachs
Coriandrum sativum	Coriander or Dhania	Umbelliferae	Leaves	Quercitin, Kaempferal, Rhamnitin	Antispasmodic, diuretic, aphrodisiac, Rheumatism, neuralgia, ulcer of mouth and throat, refrigerant.
Curcuma longa	Turmeric or Haldi	Zingiberaceae	Rhizome	Curcumin	Antioxidant and natural dye
Emblica officinalis	Indian Goosebery or Amla	Euphorbiaceae	Leaves	Phospatides	Fevers, vomiting, indigestion, diarrhea, dysentery, Ophthalmic diseases, diuretic, antiscorbutic
Eucalyptus	Eucalyptus or Neelagiree Aam	Myrtaeae	Leaves	Eucalyptol	Stimulant, antiseptic and antibacterial

BOTANICAL NAME	COMMON NAME	FAMILY	PART USED	ACTIVE COMPOUND	MEDICINAL USES
Mentha piperita	Peppermint or Pudina	Lamiaceae	Leaves	Peppermint oil	Dyspepsia, nausea, headaches and heartburn
Nerium oleander	Oleander or Kaner	Apocynaceae	Leaves, fruit	Cardenolide	Cardiotonic, diaphoretic, diuretic, expectorants
Ocimum sanctum	Tulsi	Labiatae	Leaves	Ursolic acid, Apigenin, Luteolin	Malaria, chronic fever, haemorrhage, dysentery, dyspepsia, anthelmintic, diaphoretic, anticatarrhal, Expectorant.
Withania somnifera	Ashwagandha	Solanaceae	Leaves	Withaferin A	Promote strength and vigour, skin lesions, applied on swelling, pus, ulcers, boils, hiccup, cough, dropsy, rheumatism
Zingiber officinale	Ginger or Adrak	Zingiberaceae	rhizome	Zingiver	Rheumatism, piles, pulmonary and catarrhal diseases, dropsy, toothache

2.2 Preparation of Plant Extracts

The plant extraction was carried out using known standard procedures [7]. The fresh samples of plant material were properly washed under tap water and kept in room at normal room temperature till complete dry. 5gms of dried material from each sample was ground with the help of grinder to fine powder. 40ml of distilled water was added in each fine powdered material to make the final volume 25ml, further it was divided into 3 equal parts. Each one was filtered with muslin cloth through different solvents used i.e., distilled water, acetone and ethanol. Again, Whatmann's filter paper was used for the complete filtration process. Different concentrations are made for each solvent phase viz., '0.2'-0.2ml plant extract + 0.8ml solvent, '0.4'-0.4ml plant extract + 0.6ml solvent, '0.6'-0.6ml plant extract + 0.4ml solvent, '0.8'-0.8ml plant extract + 0.2ml solvent, 'C'-Crude plant extract.

2.3 Microbial Cultures

Total three microbial strains including bacteria & fungal strains were used in this study. Microbial cultures of Gram-positive bacteria *Bacillus subtilis* (Strain no 441), Gram-negative *Escherichia coli* (Strain no 45) and fungus A*spergillus niger* (Strain no. 281) were procured from collection center, Department of Biotechnology, Meerut Institute of Engineering & Technology, Meerut. For the experimental purpose 1A, 1B and F was the nomenclature given to the *Bacillus subtilis*, *Escherichia coli* and A*spergillus niger* respectively. The presented work was carried out in the same organization.

2.4 Antimicrobial Assay of Plant Extracts

The bacterial cultures of *Bacillus subtilis* and *Escherichia coli* strains were maintained on Nutrient Agar and Eosin Methylene Blue Agar Media slants respectively. Potato Dextrose Agar (PDA) slants were used to maintain the fungal strain, stored at 4ºC. Cultures were reactivated before every test.

Activation of bacterial cultures was carried out by inoculation of culture from the slants on to Nutrient broth and for fungal strain on Potato

Dextrose Broth and then incubating them overnight at 37º C. A single colony was picked from the slants and transferred to respective broths and incubated for 16-18 hours at 37ºC prior to test.

To determine the antimicrobial activity, nine sets were prepared for each plant extract, three for gram positive, three for gram negative bacteria and three for fungal strain in all the solvent phases used for different solvent distilled water, acetone and ethanol. Spreading of microbial culture was done by 'L' shaped spreader. Five wells [8] of about 5 mm diameter were punctured and different concentrations of plant extracts were inoculated with the help of micropipette in each well. Petri plates for 24 hours at 37ºC. Diameter of zone of inhibition was measured in each set after 24 hours. After incubation the diameter of inhibitory zones formed which were measured in cm and recorded.

3. Results & Discussion:

The results of antimicrobial activity of aqueous, acetone and ethanol extracts of 16 Indian medicinal plants were investigated using agar well diffusion method against selected microbes such as *Bacillus subtilis*, *Escherichia coli* and A*spergillus niger* are shown in Table 2. All the medicinal plant extracts used against the pathogenic organisms have showed varied degree of antimicrobial activity against the microbes.

The ethanolic extract of *Aegle marmelos* leaves exhibit antibacterial activity only against *Bacillus subtilis* [9]. In *Allium cepa* the distilled water extract is ineffective against both the test bacterial species whereas the acetone and ethanolic extracts show marked inhibition against both the bacteria. The distilled water and acetone extract of garlic show marked inhibition of fungus with maximum zone of inhibition [10]. The ethanolic extract was less effective against fungi. In *Azadirachta indica* [11] and *Calotropis procera* the ethanolic extract exhibited excellent antimicrobial activity against all the microbes succeeded by acetone and then distilled water [12]. In *Cassia fistula,* the distilled water, acetone and ethanolic extracts of leaves show excellent antimicrobial activity against Gram negative bacteria i.e., *E. coli* and only distilled water and ethanolic extracts are found to be inhibitory for Gram positive bacteria i.e., *Bacillus subtilis* [13,14]. In *Coriandrum sativum,* the distilled water, acetone and ethanolic extracts of leaves are excellent inhibitors *Bacillus subtilis* while only one extract of *Coriandrum sativum* i.e., acetone extract showed inhibition of *E. coli* [15]. In *Curcuma longa*, the acetone extract shows good antibacterial activity against both the bacterial strains but distilled water extract shows inhibitory effect against *Bacillus subtilis* only [16]. In *Emblica officinalis* the acetone and ethanolic extracts of fruits show inhibitory effect against fungus while all the extracts of leaves are ineffective against fungus. The distilled water, acetone and ethanolic extracts of *Emblica* leaves show marked inhibition of bacteria [17]. In *Mentha piperita,* the distilled water, acetone and ethanolic extracts of leaves show good inhibitory effect against *B. subtilis* and *A. niger* while, the extracts are found to be ineffective against *E. coli* [18]. In *Zingiber officinalis*, the ethanolic extract exhibited good antimicrobial activity for all microbes while the distilled water and acetone extracts are ineffective against three test micro-organisms [19]. Plant extracts of *Nerium oleander* [20], *Ocimum* sanctum [21], *Withania somnifera* [22] and *Zingiber officinale* also exhibit varied degree of antimicrobial activity against the.

Table 2: Diameter of zone of inhibition (cm) of plant extracts against microorganisms.							
Plant Name	**Solvent Phase**	**Microbial Strain**	**Zone of Inhibition**				
			C	**0.8**	**0.6**	**0.4**	**0.2**
Aegle marmelos	**D/W**	**1A**	1.2	1.3	-	-	-
		1B	-	-	-	-	-
		F	-	-	-	-	-
	Acetone	**1A**	1.2	1	1	0.9	0.8
		1B	-	1.2	1	0.9	0.8
		F	2	2.1	1.8	1.7	1.5
	Ethanol	**1A**	1	1	0.9	0.9	0.7
		1B	-	0.9	0.9	1	1
		F	2	0.8	0.6	0.4	0.2
Allium cepa	**D/W**	**1A**	-	-	-	-	-
		1B	-	-	-	-	-
		F	2	2.2	2	1.7	1.3
	Acetone	**1A**	-	0.8	-	0.8	1.1
		1B	-	-	0.8	-	-
		F	-	-	-	-	-
	Ethanol	**1A**	1.2	-	-	0.6	0.9
		1B	0.7	0.8	0.8	0.5	0.6
		F	-	-	0.9	0.8	0.7
Allium sativum	**D/W**	**1A**	-	-	-	-	-
		1B	-	-	-	-	-
		F	1.3	-	2.2	2	1.2
	Acetone	**1A**	-	0.9	0.8	0.8	0.9
		1B	1.4	1.4	1.1	1.1	1
		F	1.3	1.2	-	1.1	-
	Ethanol	**1A**	-	0.9	0.9	1	1.1
		1B	1.1	1.2	0.9	0.8	1.2
		F	-	1.2	-	-	-
Azadirachta indica	**D/W**	**1A**	1.3	1.2	1.2	1	1
		1B	-	-	-	-	-
		F	-	-	-	-	-
	Acetone	**1A**	1.3	1.2	1	0.9	0.9
		1B	-	1	1	0.9	0.8
		F	2	1.8	1.7	1.3	1.5
	Ethanol	**1A**	1.2	1.2	1.1	1.3	1
		1B	1.3	1.3	1.2	1.1	0.8
		F	1.9	1.6	1.4	1	0.9

Plant Name	Solvent Phase	Microbial Strain	Zone of Inhibition				
			C	0.8	0.6	0.4	0.2
Calotropis procera	D/W	1A	-	-	-	-	-
		1B	-	-	-	-	-
		F	-	-	-	-	-
	Acetone	1A	-	-	0.8	1.2	1
		1B	0.8	0.9	-	-	0.8
		F	-	1.3	0.9	1.1	1
	Ethanol	1A	1.1	1.3	1	1.1	1
		1B	1	1	1.1	-	0.9
		F	1.2	1.2	0.9	-	-
Cassia fistula	D/W	1A	1.7	1.6	1.4	1.2	1
		1B	1.6	1.5	1.4	1.4	1.1
		F	-	-	-	-	-
	Acetone	1A	-	-	-	-	-
		1B	1.1	1.1	1	0.9	0.8
		F	1.2	1.2	1.1	1	0.8
	Ethanol	1A	1.1	1	0.9	0.9	0.8
		1B	1.1	1	0.9	0.7	0.5
		F	1.6	1.5	1.3	1.1	1.2
Catharanthus roseus	D/W	1A	-	-	-	-	-
		1B	-	-	-	-	-
		F	-	-	-	-	-
	Acetone	1A	0.9	0.8	1.1	1	0.7
		1B	1.1	1	0.9	0.8	0.7
		F	-	-	-	-	-
	Ethanol	1A	1.2	1.3	1.2	1.2	1.1
		1B	1.2	1.1	1	1	0.9
		F	1.4	1.7	1.5	1.4	1.3
Coriandrum sativum	D/W	1A	0.9	0.7	0.7	0.9	0.8
		1B	0.9	-	-	-	-
		F	1.1	1.3	1.2	1.2	-
	Acetone	1A	1.3	-	0.7	1.1	0.9
		1B	0.7	0.8	0.8	0.8	0.6
		F	-	1.2	1.5	1.3	1.5
	Ethanol	1A	0.7	-	0.8	1	0.9
		1B	-	-	-	0.8	0.7
		F	1.8	1.6	1.6	1.3	1

Plant Name	Solvent Phase	Microbial Strain	Zone of Inhibition				
			C	0.8	0.6	0.4	0.2
Curcuma longa	D/W	1A	0.9	-	-	0.8	0.9
		1B	0.8	-	0.9	-	-
		F	0.9	1.3	-	1.6	1
	Acetone	1A	1.5	1	1.4	0.7	0.8
		1B	1.2	1	1	1.2	1
		F	0.8	-	1	0.8	0.7
	Ethanol	1A	-	-	-	-	-
		1B	1.3	1	1.5	1.1	-
		F	1.3	1.4	0.9	-	0.8
Emblica officinalis	D/W	1A	1.7	1.6	1.5	1.3	1
		1B	1.8	1.7	1.6	1.5	1.3
		F	-	-	-	-	-
	Acetone	1A	2	2	1.8	1.7	1.6
		1B	2.1	2	1.9	1.7	1.4
		F	-	-	-	-	-
	Ethanol	1A	1.7	1.4	1.6	1.6	1.5
		1B	2.1	1.5	1.6	1.7	1.8
		F	-	-	-	-	-
Eucalyptus	D/W	1A	1.2	1.1	0.9	0.8	0.9
		1B	1.2	1	0.9	0.8	0.8
		F	-	1.6	1.5	1.3	1.1
	Acetone	1A	1.7	1.5	1.3	1.2	1.2
		1B	1.5	1.3	1.2	1.1	1
		F	2.1	2	1.7	1.5	1.3
	Ethanol	1A	1.4	1.3	1.2	1.1	1
		1B	1.2	1.1	1.1	1	1
		F	1.8	1.8	1.7	1.5	1.4
Mentha piperita	D/W	1A	1.5	1.5	1.3	1.2	1.4
		1B	-	-	-	-	-
		F	0.9	1.3	1.1	1.1	0.8
	Acetone	1A	-	0.9	1.2	1	1.1
		1B	-	-	-	-	-
		F	0.9	1.3	1.1	1.1	0.8
	Ethanol	1A	0.8	-	0.9	0.8	0.7
		1B	-	-	-	-	-
		F	1.2	1.3	1.5	1.1	1.4

Plant Name	Solvent Phase	Microbial Strain	Zone of Inhibition				
			C	0.8	0.6	0.4	0.2
Nerium oleander	D/W	1A	-	-	-	-	-
		1B	-	-	-	-	-
		F	-	-	-	-	-
	Acetone	1A	-	1	0.9	0.9	0.8
		1B	0.8	1.2	1.1	0.9	1.1
		F	-	-	0.8	0.7	-
	Ethanol	1A	-	-	0.8	0.8	0.8
		1B	0.8	1.2	1.1	0.9	1.1
		F	-	-	0.8	0.7	-
Ocimum sanctum	D/W	1A	-	-	-	-	-
		1B	-	-	-	-	-
		F	1.2	1.1	1	-	-
	Acetone	1A	-	-	-	-	-
		1B	1.2	0.8	1.2	0.8	1.5
		F	-	-	-	-	-
	Ethanol	1A	-	-	0.8	1	1.1
		1B	-	-	-	-	0.8
		F	1.6	-	-	-	0.9
Withania somnifera	D/W	1A	-	-	-	-	-
		1B	-	-	-	-	-
		F	-	-	-	-	-
	Acetone	1A	1.8	1.6	1.5	1.4	1.2
		1B	1.9	1.8	1.6	1.3	1.1
		F	1.5	1.3	1.2	1.1	1
	Ethanol	1A	1.7	1.5	1.4	1.3	1.2
		1B	1.9	1.7	1.5	1.4	1.2
		F	1.7	1.5	1.4	1.4	1.3
Zingeber officinale	D/W	1A	-	-	-	-	-
		1B	-	-	-	-	-
		F	-	-	-	-	-
	Acetone	1A	-	-	-	-	-
		1B	-	-	0.9	0.8	1
		F	-	-	-	-	-
	Ethanol	1A	1.1	1	0.9	0.8	0.8
		1B	1.1	1.4	1.3	1.1	1.2
		F	1.2	1.2	1.1	1.1	1

bacterial and fungal strains

Conclusion:

Plants contain diversified phytochemicals. These plants can be used for treating the various diseases as those phytochemicals have the potential to act on diversified microbial metabolic processes. The results of this work established that all the tested plant extracts have antibacterial and antifungal activity against Gram-positive bacteria *Bacillus subtilis*, Gram-negative *Escherichia coli* and fungus *Aspergillus niger*. For the future studies the other plant parts can be used to evaluate the antiviral and antiparasitic activities. The other advance studies pertaining to antimicrobial response will open the avenues to treat the various alignments.

Conflict of interest

The authors declare no potential conflicts of interest with respect to research, authorship, and/or publication of this manuscript.

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