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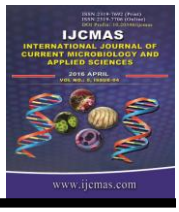

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Antibacterial Activity of Herbal Plants

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A B S T R A C T

The main objective of the study was to find the antibacterial activity of plant *Adhatoda vasica, Aegle marmelos, Aristolochia bracteolata, Cardiospermum halicacabum, Cordia myxa, Eclipta alba, Mukia maderaspatuna, Ocimum basilicum, Plectranthus ambionicus* and *Solanum xanthocarpum.* The antibacterial activity was determined by the agar well diffusion method. The antibacterial activity was against three gram positive bacteria *Bacillus subtilis, Streptococcus pyogenes, Staphylcoccus aureus* and two gram negative bacterial strains *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* by using solvent extract Acetone Aqueous and Ethanol extract. For the present study these one plant were screened to detect the presence of the active metabolites like saponins, tannins and terpenoids.

Introduction

The plant *Coleus ambionicus (*synonym: *Plectranthus ambionicus, Coleus aromaticus)* commonly known as country borage, Indian borage, is a dicotyledonous plant belonging to the family *Lamiaceae* (Warrier, 1994). It is a large succulent aromatic parennial herb. Much branched, fleshy highly aromatic pubescent herb with distinctive smelling leaves. The plant is distributed throughout India, cultivated in the gardens. It is folkloric medicinal plant used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, (Nadkarni, 1996), hiccough, bronchitis, helminthiasis, colic and covulsions and epilepsy(Gil –otaiza, 1997). It is used to treat colds and cough as well as arthritic inflammations. Indian Material medica includes about 200 drugs of natural origin almost all of which are derived from different traditional system and folklore practices (Narayana *et al.,* 1998). Antimicrobial activity of plants can be dedicated by observing the growth response of various microorganisms to those plant tissues or extracts, which are placed in them. Many methods for detecting such activity are available, but since they are not equally sensitive or even based on the same

principle, the results obtained will also be influenced by the method selected and the microorganisms used for the test.

Henna (*Lawsonia inermis L.)* is a small shrub. Frequently cultivated in India, Persia, and along the the African coast of the Mediterranean sea. Powdered leaves of this plant, in the form of a paste, are used both has a cosmetic and as a remedy for boils, wounds and some my cotic infections in certain countries of the middle east (Krithiga and Jayachitra, 2012). Allopathic treatment may either be permanent or temporary depending on the patient's health has improved through nutritional methods, sometimes they can be weaned from the syunthetic drugs (Biswas 2006). Evaluation of indian traditional medicine is possible through the proper exploiration of wide bio-diversity and great ancient treatises of traditional medicine with the light of modern tools and techniques (Mukherjee, 2002). Numerous medicinal plants and their formulation are used for disorders in the ethno medicines in India.

Materials and Methods

Collection of Plant Sample

Totally ten wound samples were collected from the government hospital outpatient at orathanadu. Samples were collected in a sterile screw cap test tubes. It was taken to the laboratory for the identification of pathogen. In case of delay the samples are kept in refrigerator in order to avoid multiplication of the normal flora.

Screening the Organisms Present in the Wound Sample

Isolation and Identification of Wound Sample

A loopful of wound sample collected from ten different outpatients were taken separately. It was streaked on the selective media such as Blood agar, Nutrient agar, Eosin methylene blue agar, MacConkey agar and Cetrimide agar and incubated at 37^0c in aseptic condition for 24 hours. After the incubation period the individual colony emerging from the medium was sub cultured separately in the same medium for identification. They are identified morphologically and biochemical methods.

Collection of Plant Sample

Fresh plants were collected randomly from Marudupandiyar college herbal garden Thanjavur of Tamilnadu, India, in January 2016. The taxonomic identifies of this plant were determined by Dr.A. Panneerselvam, Department of botany and microbiology, associate professor A.V.V.M. Sri Pushpam college, poondi Thanjavur. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Preparation of Plant Extract

Aqueous Extraction

The aqueous extractions of the water soluble ingredients were carried out using the method as described by Azusu (1986). 15g of each of the grounded leaves were extracted by successive soaking for 2 days using 35ml of distilled water in a 250ml sterile conical flask. The extracts were filtered by using Whatman filter paper No.1.The filtrates were concentrated in vacuum at 60^0c and stored in universal bottles and refrigerated at 4^0c prior to use.

Ethanol Extraction

The ethanol extractions of the active ingredient of the leaves were prepared 25 pi of the herbal plants leaves were soxlet extracted using 250 ml of 95% ethanol. The

extraction lasted for six hours. The volatile oil obtained was concentrated by evaporation using water bath at 100^0c.

Acetone Extraction

The acetone extract was prepared by suspending 100g of powdered leaves in 500ml 95% acetone. This mixture was diluted with 600ml of acetone and then allowed to stand for 24hrs. The resulting extract was decanted and filtered through a whatman filter paper. The filtrate was the concentrated with rotary evaporator at 4^0c.

Screening of Phytochemical Compounds

The various solvent extracts of the coarse powder of leaves of herbal plants were subjected to phytochemical tests for the identification of various active constituents,using the methodology followed(Malcon and Sofowora, 1969). The following major pharmaceutically valuable phytochemical compounds were analyzed.

Detection Saponin

To one ml each of the various extracts were separately mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 min. The formation of foam indicated the presence of saponins.

Detection of Terpenoids

To five ml each of various extracts were dissolved in 5 ml of chloroform separately (stock solution). Then they were subjected to Libermann-Burchard test. To one ml of each of the stock solution, a few drops of acetic anhydride and 1 ml of concentrated sulphuric acid were added from the sides of the test tubes and allowed to stand for 5 min. Formation of brown ring at the junction of two layers and the upper layer turned green indicated the presence of terpenoids.

Detection of Tannins

To five ml each of the various extracts were dissolved in minimum amount of water separately and filtered. Then filtrates were taken separately and added a few drops of aqueous basic lead acetate solution. Formation of reddish brown colour precipitate indicated the presence of tannins.

Test Organism

Test bacterial strain was grown in nutrient broth for 18-24hours at 37^0c on rotary shaker. Cells were kept at 4^0c.

Antibacterial Susceptibility Testing

Antibacterial activity of agar well diffusion method:

In the agar well diffusion inhibition test as described by Opara and Ansa, 1993,0.2ml of a 24hr broth culture of the bacteria was aseptically introduced and evenly spread using bent. Sterile glass rod on the surface of gelled sterile Muller-Hinton agar plates. Three wells about 6.0mm diameter were aseptically punched on agar -plate using a sterile cork bore allowing at least 30mm between adjacent wells and between peripheral wells and the edge of the petridish. Fixed volumes (0.2 ml) of the leave extract were then introduced into the wells in the plates. A control well was in the centre with 0.01 ml of the extracting solvent. The plates incubated at 37^0c for 24hr for the test bacteria. The plates were duplicated in all the experiments.

Results and Discussion

The isolated organisms like *Staphyloccocus aureus, Bacillus subtilis, Streptococcus pyogenes, Pseudomonas aeruginosa, Klebsiella pneumoniae* exhibited the antibacterial activity for three solvents

extracts acetone, aqueous and ethanol of herbal plants

Acetone Extract

Acetone extract of *Adhatoda vasica, Solanum xanthocarpum, Plectranthus ambionicus, Cordia myxa, Cardiospermum halicacabum, Ocimum basilicum, Eclipta alba, Mukia maderaspatuna* showed highest activity 15mm,12.5mm,12mm against *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Klebsiella pneumoniae* and *Pseudomonas aeruginosa.*

Ethanol Extract

The ethanol extract of *Plectranthus ambionicus, Solanum xanthocarpum, Cardiospermum halicacabum, Mukia maderaspatuna, Adhatoda vasica, Aristolochia bracteolata, Ocimum basilicum* and *Eclipta alba* Showed maximum highest activity 17.5mm,15mm,14mm,12.5mm against *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtlis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

Aqueous Extract

The aqueous extract of *Aegle marmelos, Cordia myxa, Eclipta alba,Cardiospermum halicacabum, Solanum xanthocarpum, Plectranthus amboinicus* maximum activity 12.5mm, 12mm,10mm and 9mm against *Streptocoocus pyogenes,Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus aureus.* The least activity 5mm and 3mm against *Bacillus subtilis.*

The antibacterial activity of selected ten plants ethanol, acetone and aqueous extracts was teste against the commonly acquired clinical pathogen of *Bacillus subtilis, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes.* The sensitivity test is done to determine the degree of sensitivity or resistance of the selected gram positive and gram negative pathogens toward the antimicrobial drugs.

Pseudomonas aeruginosa has shown the maximum zone of inhibition of 17.5mm at the highest concentration of 200 µl, which proves that *P.aeruginosa* is susceptible toward the ethanolic extract of *Adhatoda vasica* which is significant to the research conducted by (Prasannabalaji, *et al., 2012).* *Mukia maderaspatuna* also shown zone of inhibition on *Bacillus subtilis* at the concentration of 100 µl. Since the inhibition zone is up to 14mm, it shows that *B.subtilis* is susceptible toward *Mukia maderaspatuna.* The *Klebsiella pneumoniae* is concluded to be resistant toward *Mukia maderaspatuna* since the maximum inhibition zone is only 12mm even at the highest concentration.In a previous study, the ethanol extract of *P.ambionicus* showed a moderate antibacterial activity toward all the selected gram positive and gram negative bacteria (Uma Saraswati, *et al.*,2012*).* Similarly, acetone extracts of *Mukia maderaspatuna* also shows inhibitory effect on the *S.aureus* with maximum zone of inhibition 15mm at the highest concentration of 200 µl.In the present study the ethanolic extract of *Aristolochia bracteolata* has shown a less antibacterial activity against both *Bacillus subtilis* and *Pseudomonas aeruginosa.* This result is supported by the study conducted by (Chirag Modi *et al.,* 2012). Where it was stated that all the tested gram negative bacteria including *P.aeruginosa* and *Klebsiella pneumoniae* showed zone of inhibition against acetone extract of *Mukia maderaspatuna.*

Biochemical Test

		Organisms				
S.No	**Test**	***S.aureus***	***S.pyogenes***	***K.pneumoniae***	***B.subtilis***	***P.aeruginosa***
1.	**Gram strains**	**+ve**	**+ve**	**_ve**	**+ve**	**_ve**
2.	**Shape**	**Coccus**	**Coccus**	**Rod**	**Rod**	**Rod**
3.	**Motility**	**_ve**	**Non motile**	**Motile**	**+ve**	**Motile**
4.	**Indole**	**_ve**	**_ve**	**+ve**	**+ve**	**_ve**
5.	**MR**	**_ve**	**+ve**	**_ve**	**_ve**	**+ve**
6.	**VP**	**_ve**	**+ve**	**+ve**	**_ve**	**_ve**
7.	**Citrate**	**+ve**	**+ve**	**_ve**	**+ve**	**+ve**
8.	**Urease**	**+ve**	**_ve**	**_ve**	**_ve**	**_ve**
9.	**Catalase**	**+ve**	**+ve**	**+ve**	**+ve**	**_ve**
10.	**Oxidase**	**_ve**	**_ve**	**_ve**	**_ve**	**+ve**
11.	**TSI**	**A/K/ gas _ve**	**A/K/gas_ve**	**A/K/gas+ve**	**A/A/gas+ve**	**A/K/gas_ve**

Some Selected Medicinal Plants used in the Antibacterial Activity

Vernacular name	**Scientific name**	**Family**	**Parts**	**Uses**
Adhatoda	*Adhatoda vasica*	Acanthaceae	Leaves	Whooping cough,chronic bronchitis
Kayyanthara	*Eclipta alba*	Compositae	Leaves	Rheumatic joint pain,digestion,enlarged spleen
Aduthinapalai	*Aristolochia bracteolata*	Aristolochiaaceae	Leaves	Puragative,anthelmintic
Kandakathri	*Solanum xanthocarpum*	Solanaceae	Leaves	Gonorrhoea,cough,sore throat,rheumatism
Omavalli	*Plectranthus ambionicus*	Lamiaceae	Leaves	Hiccough,epilepsy,chronic asthma
Mosumosukkai	*Mukia maderaspatuna*	Curcurbitaceae	Leaves	Vertigo,biliousness,scabies
Vilva maram	*Aegle marmelos*	Rutaceae	Leaves	Constipation,diarrhoea,dysentry
Modakathan	*Cardiospermum halicacabum*	Sapindaceae	Leaves	Snake bites,itchy skin,ear ache
Vilva ilai	*Cordia myxa*	Boraginaceae	Leaves	Ulcers and head ache
Thiruneetrupachilai	*Ocimum basilicum*	Labiatae	Leaves	Insect stings,snake bites and skin infections

Phytochemical Analysis

Plant name	***Saponins***			***Tannins***			***Terpenoids***		
	Acetone	Aqueous	Ethanol	Acetone	Aqueous	Ethanol	Acetone	Aqueous	Ethanol
Aristolochia bracteolata	-	-	-	+	+	-	++	-	-
Solanum xanthocarpum	-	-	-	-	++	-			
Ocimum basilicum	-	-	-	+	++	-			
Crdiospermum halicacabum	-	-	-						
Eclipta alba	-	-	-						
Cordia myxa	-	-	-				++	+	-
Aegle marmelos	-	-	-						

Antibacterial Activity of Selected Herbal Plants

Plant name		Inhibition of growth (Diameter in mm)		
		Acetone	**Ethanol**	**Aqueous**
Streptococcus pyogenes	*Aegle marmelos*	-	-	12.5mm
	Cordia myxa	10mm	10mm	5mm
	Plectranthus ambionicus	10mm	7mm	7mm
	Adhatoda vasica	-	-	-
	Solanum xanthocarpum	12.5mm	5mm	10mm
	Mukia maderaspatuna	10mm	15mm	-
	Cardiospermum halicacabum	7mm	6mm	12mm
	Eclipta alba	10mm	6.3mm	10mm
	Ocimum basilicum	-	15mm	-
	Aristolochia bracteolate	-	-	-
Pseudomonas aeruginosa	*Aegle marmelos*	7.5mm	6mm	5mm
	Cordio myxa	5.5mm	9mm	5mm
	Plectranthus ambionicus	7mm	5.5mm	6.5mm
	Adhatoda vasica	6mm	17.5mm	-
	Solanum xanthocarpum	5.5mm	-	6mm
	Mukia maderaspatuna	-	7mm	7mm
	Cardiospermum halicacabum	7.5mm	10mm	7.5mm
	Eclipta alba	10mm	2.5mm	8mm
	Ocimum basilicum	10.5mm	6.5mm	-
	Aristolochia bracteolate	-	7.5mm	6mm
Bacillus subtilis	*Aegle marmelos*	-	3.5mm	5mm
	Cordio myxa	-	10mm	-
	Plectranthus ambionicus	-	12mm	-
	Adhatoda vasica	11mm	-	-
	Solanum xanthocarpum	6mm	3mm	-
	Mukia maderaspatuna	-	14mm	-
	Cardiospermum halicacabum	6.5mm	7mm	-
	Eclipta alba	-	-	3mm
	Ocimum basilicum	9.5mm	-	-
	Aristolochia bracteolate	4mm	-	-
Klebsiella pneumoniae	*Aegle marmelos*	6mm	8mm	12mm
	Cordia myxa	5mm	8mm	12mm
	Plectranthus ambionicus	7mm	8mm	-
	Adhatoda vasica	7mm	3mm	3.5mm
	Mukia maderaspatuna	15mm	12mm	-
	Cardiospermum halicacabum	-	5mm	-
	Eclipta alba	10mm	10.5mm	-
	Ocimum basilicum	6.5mm	8mm	-
	Aristolochia bracteolate	10mm	5mm	-
Staphylococcus aureus	*Aegle marmelos*	7mm	6.5mm	8mm
	Cordia myxa	6.5mm	10mm	5mm
	Plectranthus ambionicus	10mm	6mm	9mm
	Adhatoda vasica	8mm	10mm	-
	Solanum xanthocarpum	5mm	11mm	6mm
	Mukia maderaspatuna	15mm	7mm	-
	Cardiospermum halicacabum	12mm	10mm	7mm
	Eclipta alba	6.3mm	8mm	2mm
	Ocimum basilicum	10.5mm	12mm	-
	Aristolochia bracteolate	-	5mm	-

The presents study revealed that the acetone and ethanolic extract of both herbal plants has antibacterial activity against the common clinical pathogen of *Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus* and *Streptococcus pyogenes.* However both plants show higher antibacterial activity in *Pseudomonas aeruginosa,Klebsiella pneumoniae* and *Bacillus subtilis.* This proves that the leave extract of herbal plants higher inhibitory effect on gram positive bacteria (*Staphylococcus aureus* and *Streptococcus pyogenes)* compared togram negative bacteria (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae).*This finding is supported by previous research studies where it was reported that the plant extract has higher potential to inhibit gram positive bacteria compared negative (Basari and Fan, 2005).Gram negative bacteria are more resistant to plants extract compared to gram positive bacteria similar to the study of (Archana *et al.,* 2012). However, studies *Adhatoda vasica* has higher antibacterial activity toward gram positive and gram negative bacteria. According to Mihaela, gram positive bacteria have lack of additional permeability barrier compared to gram negative which makes it more susceptible toward the plant extracts (Mihaela Marilena, 2010).

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