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RESEARCH ARTICLE

Antimicrobial Activity of the Extract of Stem Bark of Diospyrosebenum

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ABSTRACT
In this study, the ethanol extract of D. ebenum was tested for its antimicrobial activity against strains gram-positive and gram-negative. A total of 5 Gram –ve and 5 Gram +ve were used in this study Salmonella sps., E. colis, Pseudomonas sps., Bacillus subtilis and Staphylococcus aureus, Diospyrosebenum, Bacillus pumilus, Staphylococcus aureus, Staphylococcus albas. In order to determine the minimal inhibitory concentration, assays were carried out by micro dilution method. The extract was screened for antimicrobial activity, and it showed antibacterial activity.
Keywords: Antimicrobial activity, Diospyrosebenum, Escherichia coli, Ebenaceae, Pseudomonas aeruginosa

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CONTENTS

1. Introduction

The history of plants being used for medicinal purpose is probably as old as the history of mankind. India is a nation blessed with rich heritage of traditional systems of medicine and rich biodiversity with 15 agro-climatic zones. [1] Wild plant species are excellent source of wide range of biochemicals. Indian medicinal plants have been used to treat diseases for thousands of years. Medicinal plants constitute the main source of new pharmaceuticals and health care products. [2] The use of medicinal plants in industrial societies is mainly for extraction and to obtain several drugs from these plants. Extraction and characterization of several active phytocompounds from these green factories have produces high activity profile drugs. [3] It is believed that crude extract in medicinal

plants are biologically more active than isolated compounds due to their synthetic effects. [4] Secondary metabolites of plants having the defense mechanism against predation by many micro organisms, insect and herbivores. [5] Secondary metabolites such as alkoloids, flavonoids, tannins and phenolic compounds have been established as the bioactive compounds of plants. [6] Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available with less side effects. The aim of the present study is to screen the phytochemicals in the bark extracts and test antibacterial activity of of Stem Bark of Diospyrosebenum.

2. Materials and Methods

Preparation of Crude Extracts of Plant Material:

The extracts were prepared from air-dried Bark of *Diospyrosebenum.* About 25 *g* of the powdered each plant material was taken in a dry 250 ml conical flask, and then 100 ml 95% ethanol was added and allowed to macerate over night. The next day the mixture was vigorously stirred for 10 minutes and allowed to settle. The supernatant liquid was filtered using Whatman No.1 filter paper. The residual plant material was extracted twice using 100 ml 95% ethanol. The filtrates were combined and the solvent was evaporated in vacuum using rotatory evaporator. The residual plant material was dried well and again extracted with water, ethyl acetate using the same procedure as above. The crude extracts were kept at 4°C.[2-5]

Bacterial strains:

Gram Positive Bacteria

Bacillus subtilis, Diospyrosebenum,
Bacillus pumilus, Staphylococcus
aureus, Staphylococcusalbas.

Gram Negative Bacteria

Escherichia coli, Klebsiella sps.,
Salmonella sps., Shigella sps,
Pseudomonas sps., Proteussps.

Cup-plate agar diffusion method using Nutrient agar

The antibacterial activity of ethanol extract of the plant against 10bacteria (5-Gram +ve and 5-Gram -ve) was evaluated by using agar well diffusion method

Materials used:

Nutrient broth (Himedia), Nutrient agar (Himedia),18-24 hrsculture, Sterilepetridishes, Sterilemicropipettes, Sterile cottonswabs, Sterilecorkborer, Sterile testtubes.

Preparation of Nutrient broth:

Nutrient broth - 3.8gm
Distilled water - 100ml

Above components were dissolved in 100 ml distilled water and pH was adjusted to 7.2. This solution was sterilized by autoclaving at15 lbs/121^{o}C for 20minutes.

Preparation of Inoculums:

One day prior to these testing, inoculations of the above bacterial cultures were made in the nutrient broth and incubated at 37^{0}C for 18 – 24 hrs.[6-9]

1.2.4 Preparation of Medium (Nutrient agar):

Nutrient agar - 2.8gm
Distilled water - 100ml

The agar was dissolved in to distilled water and pH was adjusted to 7.4+0.2. It was sterilized by autoclaving at 15lbs/121^{o}C for 20 minutes.

1.2.5 Preparation of test solutions:

Each test compound (95%Ethanolic extract which contain 5 mg) was dissolved in sterile water (5 ml) to give stock solution of concentration 1000µg /ml. Then 0.1 ml of this solution was used for testing.

1.2.6 Preparation of standard solution:

Standard drug Ampicillin and Penicillin were used. The concentration was 100µg/ml.

1.2.7 Method of testing:

Nutrient agar plates were prepared by pouring 15 – 20 ml of the medium in to each sterilized petridish and were allowed to set at room temperature. The cell suspension was standardized to the density of 530 nm using spectrophotometer and was inoculated over the surface of agar medium using sterile cotton swab. The four cups were scooped in each plate using a sterile cup borer of 8 mm diameter. Then the solutions of test compounds (0.1 ml) were added in cups by using micropipettes and these plates were incubated at 37^{0}C for 48 hrs. The zone of inhibition was measured in mm for each organism. (these processes were performed with standard as well as with plant extract and results were shown in Table-1 and2.)

Sensitivity test:

For Standard drug

Prepare different dilution of Ampicillin /Penicillin in N-broth tubes. Add 0.1ml of microbial culture in all tubes.Mix all the tubes properly. Incubate at370C for 24 hours. Observe the growth of microbes in terms of turbidity. Ampicillin / Penicillin Stock solution 200 unit/ml. Test organism: *Bacillus subtilis, Diospyrosebenum, Bacillus pumilus, Staphylococcus aureus, Staphylococcus albas. Escherichia coli, Klebsiellasps., Salmonella sps., Shigellasps, Pseudomonas sps., Proteussps.* (+) Sign for growth observed and (-) Sign for No growth observed. *Positive control- 5 ml N-broth+0.1 ml microbial culture.**Negative -5 ml N-broth+0.1 ml heat killed microbial culture. Same procedure was performed with plant extract. (Results were shown in the Table -3 and 4).

3. Results and Discussion

Table 1: Antibacterial Activity of Antibiotics (Ampicillin and Penicillin)

Micro-organism	**Mean Diameter Inhibition Zone Inhibition**							
	Ampicillin				***Penicillin***			
	25µg/ml	50µg/ml	75µg/ml	100µg/ml	25µg/ml	50µg/ml	75µg/ml	100µg/ml
Bacillus subtilis	4mm	8mm	20mm	22mm	4mm	8mm	16mm	24mm
Diospyrosebenum	4mm	8mm	12mm	25mm	2mm	4mm	8mm	12mm

Bacillus pumilus	8mm	18mm	28mm	31mm	2mm	4mm	6mm	10mm
Staphylococcus aureus	10mm	20mm	25mm	30mm	2mm	5mm	8mm	10mm
Staphylococcus albas	8mm	15mm	20mm	25mm	2mm	6mm	9mm	11mm
Escherichia coli	15mm	30mm	45mm	50mm	15mm	30mm	45mm	50mm
Klebsiellasps.	10mm	20mm	30mm	40mm	10mm	20mm	30mm	40mm
Salmonella sps.	10mm	20mm	25mm	35mm	8mm	16mm	20mm	22mm
Shigellasps.	12mm	24mm	32mm	45mm	10mm	20mm	25mm	35mm
Pseudomonas sps.	10mm	20mm	30mm	38mm	10mm	16mm	25mm	32mm
Proteus sps.	12mm	26mm	40mm	45mm	12mm	24mm	35mm	45mm

Table 2: Antibacterial Activity of Ethanolic Extract Medicinal Plants

Micro-organism	***Diospyrosebenum***			
	25µg/ ml	50µg/ml	75µg/ml	100µg/ml
Bacillus subtilis	1mm	2mm	2mm	2mm
Bacillus mycoids	1mm	2mm	4mm	4mm
Bacillus pumilus	0mm	0mm	1mm	1mm
Staphylococcus aureus	0mm	2mm	2mm	3mm
Staphylococcus albas	0mm	1mm	2mm	2mm
Escherichia coli	0mm	0mm	1mm	2mm
Klebsiellasps.	0mm	0mm	0mm	1mm
Salmonella sps.	1mm	1.5mm	2mm	2mm
Shigellasps.	1mm	2mm	2mm	3mm
Pseudomonas sps.	1mm	2mm	3mm	3mm
Proteus sps.	2mm	2mm	3mm	4mm

Table 3: MIC of Standard Antibiotics against Gram +ve and Gram –ve Bacteria

Micro-Organism	**Amp./Peni.**	**Minimum Inhibitory Concentration (µg/ml)(conc.seraially reducing from 1 to 6)**					
	2 0 0	1	2	3	4	5	6
Bacillus subtilis		-/-	-/-	-/-	+/+	+/+	+/+
Diospyrosebenum		-/-	-/-	-/-	-/-	-/+	+/+
Bacillus pumilus		-/-	-/-	-/-	+/-	+/+	+/+
Staphylococcus aureus		-/-	-/-	-/-	-/-	+/+	+/+
Staphylococcus albas		-/-	-/-	-/-	-/-	+/+	+/+
Escherichia coli		-/-	-/-	-/-	-/+	-/+	+/+
Klebsiellasps.		-/-	-/-	-/-	-/-	+/+	+/+
Salmonella sps.		-/-	+/+	+/+	+/+	+/+	+/+
Shigellasps.		-/-	-/-	-/-	-/+	-/+	-/+
Pseudomonas sps.		-/-	-/-	-/-	+/+	+/+	+/+
Proteus sps.		-/-	-/-	-/-	+/-	+/+	+/+

(+) Sign for growth observed.

(-) Sign for No growth observed.

*Positive control- 5 ml N-broth+0.1 ml microbial culture.

**Negative -5 ml N-broth+0.1 ml heat killed microbialculture. Amp.(Ampicillin) Peni. (Penicillin)

Table 4: MIC of *Diospyrosebenum Ethanolic* extract against Gram +ve and Gram –ve Bacteria

Micro-Organism	**ETDE**	**Minimum Inhibitory Concentration (µg/ml) (conc.seraially reducing from 1 to 6)**					
		1	2	3	4	5	6
Bacillus subtilis		-	+	+	+	+	+
Diospyrosebenum		-	+	+	+	+	+
Bacillus pumilus		-	+	+	+	+	+
Staphylococcus aureus		-	+	+	+	+	+

Staphylococcus albas	-	-	-	+	+	+
Escherichia coli	-	+	+	+	+	+
Klebsiellasps.	-	+	+	+	+	+
Salmonella sps.	-	+	+	+	+	+
Shigellasps.	-	+	+	+	+	+
Pseudomonas sps.	-	+	+	+	+	+
Proteus sps.	-	+	+	+	+	+

(+) Sign for growth observed.

(-) Sign for No growth observed.

*Positive control- 5 ml N-broth+0.1 ml microbial culture.

**Negative -5 ml N-broth+0.1 ml heat killed microbial culture.

4. Conclusion

Antimicrobial activities *in vitro* of the aqueous and 95% ethanolic crude extracts of *Diospyros* was investigated against gram +ve and gram -ve bacteria by agar disc diffusion method. To find out the growth of micro-organism, we had used two types of inhabitants like Antibiotic (Ampicillin and Penicillin) and ethanolic extracts of medicinal plants of family *Diospyrosebenum (Family: Ebenaceae ,* their comparative results for antibacterial activity was discussed as per available in recorded data.(Table-1&2) .When *Diospyrosebenum was* used as 25µg/ml then it was observed that micro-organism like *Bacillus pumilus, Bacillus mycoids, E.coli, Klebsiellasps., Salmonella sps., Staphylococcus albas, Staphylococcus aureus* and *Shigellasps.* had minimum/nil zone of inhibition while with increase in concentration of active ethanolic extracts the zone of inhibition increases it was maximum i.e. 4 mm with the higher concentration i.e.75 µg/ml and 100 µg/ml in the interaction of *Diospyrosebenum.* The air dried bark powdered coarsely100 mesh of *Diospyrosebenum* extracted with 95% ethanolic and the residues were recovered using a evaporations. The ethanolic extracts was further subjected to the broth micro dilution method to determine MIC. The maximum activity was observed against bacteria namely *Diospyrosebenum* with the 100 µg/ ml.

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