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Phytochemical Analysis and Antibacterial Activities of *Boswellia Ovalifoliolata*

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Abstract: ***Different parts of Boswellia Ovalifoliolata were collected from different localities of Udayagiri hills of Velugonda hill range, Eastern Ghats, Nellore Dt, Andhra Pradesh, India. The methanolic extracts of various parts of the plants were qualitatively screened for phytochemicals using standard procedures which revealed the presence of various important bioactive chemical entities. Antibacterial activity of the methanolic extracts of stem bark and gum were evaluated against Bacillus subtilis, Staphylococcus aureus, and Gram negative bacteria Proteus vulgaris, Pseudmonas aeruginosa. The methanolic extracts of these plant parts have exhibited significant broad spectrum antibacterial activities.***

Keywords: Phytochemicals, Antibacterial, Ethnobotanical

1. Introduction

The treasure of traditional medicine, the plant kingdom has components of therapeutic values. More than 80 percent of the global population depends on the traditional medicine for improvement health, resistance and maintenance of good health. These Phytochemicals extracted from various parts of the plants act as natural antimicrobial agents. The metabolites extracted from the plants have curative properties and they can be used to treat outrageous infections and chronic diseases. One of the emerging global threats is multidrug resistance in pathogens. Hence the need of the hour is to search for novel antimicrobial agents. An abundant research work is going on to investigate the antimicrobial activity of various plant parts. A large number of antimicrobial chemical components of medicinal plants were identified and now they are under usage for treating offensive microbial infections and chronic diseases.

The Gum and stem bark of *Boswellia Ovalifoliolata* a member of Burseraceae have several therapeutic values. Oleo gum resin is used against ulcers, dysentery, joint pains and inflammations. Gum is used externally to cure arthritis (Y,N) (Nagaraju, 1992 ; Vedavathy, 1992). Gum with the fresh leaf juice is given in ulcers in mouth and throat (Vedavathy, 1992). Shade dried gum is powdered and dissolved in water. The extract obtained is mixed with curd and given orally to cure amoebic dysentery (Nagaraju, 1992). Gum powder of the plant is mixed with white precipitate of pounded stem of *Tinospora cordifolia* and honey , given orally in small quantity (10 ml),two times a day to cure hydrocele (I) (Vedavathy *et al.,* 1997a,b). Gum powder of *Boswellia ovalifoliolata* and *Boswellia serrata* and the fruit powder of *pedalium murex* are made into a paste. This is applied externally on the affected part of the testicles to cure hydrocoel . The treatment is continued for a fort-night (Y,Ye,I).

Stem bark and resin are shade dried and made into powder. About one tea spoonfuls of the mixture is given daily with sour milk on empty stomach for a month to cure stomach ulcers (Y,I).

2. Materials and Methods

The collected plant materials were cleaned, shade dried, powdered coarsely in a blender and then stored in air-tight containers for future use. The methanolic extracts of various parts of the plants were qualitatively screened for phytochemicals using standard procedures.

Fluorescence analysis was carried out following Chase and Pratt (1949) and Kokoski *et al.* (1958). The selected powdered drugs were studies both in visible light and Ultra-Violet light for their fluorescence characters.

The Pharmacognostical study includes morphology of the plant, macro and microscopical studies of the drug. Plant materials like gum and stem bark were collected for the studies. Following the techniques of Johansen (1940), Wallis (1967) and Trease and Evans (1985) the parts of the plants used as drug (stem bark, gum) were fixed in 70% alcohol for 24 hours. Free hand sections taken were stained with safranin and light green and the permanent slides were prepared. Histochemical tests were carried out by treating the sections with solutions of phloroglucinol, chloral hydrate and hydrochloric acid as per Trease and Evans (1985). Maceration was carried out for the study of various cells and tissues. The small pieces of the materials were macerated with nitric acid and potassium chloride as per Trease and Evans (1985). The measurements of tissues (Quantitive microscopy) were carried out using ocular and stage micrometers (Erma-Japan) following Wallis (1967).Line drawing were drawn by using a Camera Lucida (mirror type). Photographs of the plants and the crude drug were taken by using a Minolta camera.

3. Experimental Studies

Alkaloids test: The methanolic extract was evaporated to dryness and the residue obtained was digested with 1% Hydrochloric acid. The resulting acidic solution was divided into two parts. To one part was added Mayer's reagent and to the second part dragendorff's reagent. Appearance of precipitate or turbidity indicates the presence of alkaloids.

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Preparation of reagents

a) **Mayer's reagent:** 1.3 g of Mercuric chloride and 5g of Potassium iodide were dissolved separately in 60 ml and 10 ml of double distilled water and both the solutions were mixed and diluted to 100 ml.
b) **Dragendorff's reagent:** 8g of the Bismath nitrate was dissolved in 20 ml of conc. nitric acid and 27.2g of Potassium iodide in 50 ml of double distilled water. Both the solutions were allowed to stand till KIO_3 crystallized out. Supernatant was decanted and final volume was adjusted to 100 ml.
c) **Flavonoids:** The plant extract was tested for flavonoids by shinoda's reaction. To a few ml of Methanolic extract, conc. Hydrochloric acid, Magnesium powder and a few fragments of Mg metal was added. The presence of flavonoids was identified by the development of pink or magneta or red coloured flame.
d) **Terpenoids (Liebermann-Burchard's test):** The Liebermann-Burchard's reaction was carried out by adding 0.5 ml of H_2SO_4 along the side of the test tube containing a mixture of Methanolic HCl and Acetic anhydrate (0.5 ml each). Formation of colour from green to bluish-green (sometimes via red or blue) indicate the presence of terpenoids.
e) **Steroids (Salkaowski reagent):** Development of wine red colour on adding con. H_2SO_4 and $CHCl_3$ to the Methanolic extract indicate the positive test for steroidal compounds.
f) **Lignins:** The plant extract was tested for the presence of lignins by adding conc. HCl and 2% Forfuraldehyde. Development of red colour indicates the presence of lignans.
g) **Indoles:** The development of voilet colour in the methanolic extract on adding Ehrilich reagent is considered as a positive reaction test.
h) **Carbohydrates (Molisch test):** To the methanolic extract, α-napthol solution (1.0g dissolved in 100 ml of each ethanol w/v) was added. Then Conc. H_2SO_4 was added gentlyalong the walls of the inclined test tube. Formation of a red to voilet colour indicate the presence of carbohydrates.
i) **Test for reducing sugars:** 2ml to 5ml of Benedict's reagent, 5ml of the test solution was added. The tubes were incubated in boiling water on water bath for 10-30 minutes. The development of an orange red precipitate indicate the presence of reducing sugars.
j) **Protiens (Million's test):** Million's reagent (solution of Mercuric nitrate in nitric acid) was added to 20 ml of boiling Methanolic extract. Formation of white precipitate that gradually turns red upon heating was observed for the presence of proteins.
k) **Phenolic compounds:** To the test solution 1-4 drops of 1% Ferric chloride was added. Appearance of intense colour in the extract was observed for the presence of phenolic compounds.
l) **Anthocyanidins:** The formation of red or purple colour with the plant extract, on adding equal volume of Methanolic HCl was taken as a positive reaction for anthocyanidins.
m) **Anthraquinones:** 20ml of benzene was added to 5g of plant powder and filtered. To the filtrate 5ml of 10% Ammonium hydroxide solution was added and shaken well. The formation of pink, red or violet colour in the ammonical phase indicate the presence of anthraquinones (Wall *et al.,* 1954 anf Frans Woath, 1966).
n) **Saponin test:** The plant extract was evaporated to dryness. Tap water was added and shaken vigorously in a graduated cylinder for 15 minutes. Formation of a persistent foam was taken as a positive reaction test.
o) **Tannins:** The Methanolic extract was concentrated and the residue was dissolved in water and tested with 1% Gelatin solution and 1% Gelatin salt solution (1g gelatin dissolved in 10g NaCl w/w) to separate volumes. The appearance of white precipitate indicate the presence of tannins.

Preliminary phytochemical screening of Stem bark and gum of *Boswellia Ovalifoliolata* (Methanolic Extract)

Compound	Stem Bark	Gum
Alkaloids	-	+
Flavonoids	+	+
Indoles	+	-
Leucoantho-cyanins	+	-
Steroids	+	-
Carbohydrates	+	+
Phenols	+	+
Steroidal nucleus	+	-
Saponins	+	+
Tannins	+	+
Proteins	+	-
Lignins	+	+
Methylenedioxy functional compounds	-	-

4. Screening for Selective Secondary Constituents

A. Phenolic compounds:

a) Extractive procedure:

The phenolic constituents were extracted by following the method given by Bate Smith (1954) and Ibrahim and Towers (1960). About 25g of healthy plant material was macerated with 100ml of 2N Hydrochloric acid.(HCL). The homogenate was digested on a boiling water bath for about half-an-hour. The contents were cooled and filtered through Whatman No.1 filter paper. The filtrate was extracted repeatedly with peroxide free diethyl ether. All the extracts were pooled and concentrated to 100ml and was treated three times with 25ml of 5% anhydrous sodium carbonate solution. The pooled carbonate solution was adjusted to pH 2.0 with Conc. Hydrochloric acid.

The acidified fraction at pH 2.0 was extracted with equal volumes (25ml) of fresh diethyl ether thrice. The combined other extracts were washed with distilled water repeatedly till all traces of hydrochloric acid was removed. The ether soluble water was removed by freezing the extract. The ether was evaporated to dryness on water bath at 98^0C and the resulting phenolic compound residue was dissolved in 1ml of 95% ethanol. This was stored at low temperature in dark container for ready use.

b) Identification

About 2g of fresh weight equivalent to the final alcoholic extract was spotted on 23x29cm whatmann No.1

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Chromatographic filter paper with the help of micro pipette. The origin of the spot area was dried immediately with the help of hair-drier. Two dimensional ascending Chromatographic technique was adapted with benzene-acetic acid-water (60:70:30 v/v/v, upper layer) in the direction and sodium formate-formic acid-water (10:1:200 w/v/v) in the second direction. The Chromatographic chambers were saturated with the above solvent systems one day before the development of the Chromatograms at 22 to 24^0c. The sheets after development were removed from the chambers and dried at room temperature. The dried sheets were observed under Ultra violet light and flourescent regions were marked. The papers while exposing to Ammonia vapours were also observed under UV light and new flourescent spots were marked.

The seperated phenolic compounds on the Chromatograms were identified by comparision of R_f values and individual spot colours of Chromogenic sprays. The final confirmation was made with authentic samples by co-chromatography.

c) Chromogenic spray reagents:

i) Diazotized sulphanilic acid reagent:
25ml of freshly prepared 5% sodium nitrate was slowly added to 5ml of sulphanilic acid solution (900mg of sulphanilic acid was dissolved in 9ml of Conc. HCl and the solution was diluted to 100ml with H_2O) at 0^0c. To intensify the colours of the developed spots, 20% Sodium Carbonate solution was sprayed to the wet sheets (sprayed at 0^0c immediately after spraying the above solution).

ii) Diazotized *p*-Nitraniline reagent:
10ml of *p*-nitraniline reagent (1.5g of *p*-nitraniline was added to 45 ml of Conc. HCl and was made up to 950ml with distilled water) was mixed with 0.2ml of 5% Sodium nitrate and 10ml of 10% Sodium carbonate solution.

Table: Phenols

Compound	Stem bark	Gum
Digallic acid	-	-
Gallic acid	+	+
Ellagic acid	-	-
Aesculetin	-	-
Cis-p-coumaric acid	-	-
iso-chlorogenic acid	-	-
Chlorogenic acid	+	+
Caffeic acid	-	-
Protocatechuic acid	+	-
Gentistic acid	+	+
Scopoletin acid	+	-
Phloretic acid	+	+
ρ-Hydroxy benzoic acid	+	+
α-Resorcyclic acid	-	-
β-Resorcyclic acid	+	-
trans-ρ-coumaric acid	+	-
Vanillic acid	+	+
P-coumarylquinic acid	-	-
Cis-p-coumaric acid	+	-
Meliotic acid	+	+
Cis-Ferulic acid	+	-
Trans-Ferulic acid	-	-
Coumarin	+	+
Salycyclic acid	+	+
Cinnamic acid	+	+
Syringic acid	-	-

Table: R_f values and colour reactions of Phenolic compounds on Paper Chromatograms

Compound	R_f values in solvent		U.V fluorescence		Silphanilic Reagent	P-Nitraniline Reagent
	1	**2**	**Without NH_3**	**With NH_3**		
Digallic acid	0.3	0.7	None	Blue violet	Violet	Violet
Gallic acid	0.5	0.7	violet	Violet	Violet	Violet
Ellagic acid	0	0.3	None	Blue green	Blue green	Blue green
Aesculetin	0.3	0.3	white blue	Very bright blue	Brown	Grey
Cis-p-coumaric acid	0.4	0.8	Light blue	Deep blue	Dark brown	Blue
iso-chlorogenic acid	0	0.3	Faint blue	Duckegg green	Light orange	Brown
chlorogenic acid	0	0.8	Faint blue	Duckegg green	Orange	Brown
Caffeic acid	0.1	0.4	Blue	Bright blue	Buff	Light brow
Protocatechuic acid	0.1	0.6	None	None	Buff	Light brown
Gentistic acid	0.1	0.7	Bright blue	Yellow	Buff	Blue white
Scopoletin acid	0.3	0.5	Bright blue	Very bright blue	Yellow	Brown
Phloretic acid	0.7	0.8	None	None	Yellow	Purple white
ρ-Hydroxy benzoic acid	0.4	0.7	None	None	Bright yellow	Light pink
α-Resorcyclic acid	0.2	0.8	None	None	Brown	Orange brown
β- Resorcyclic acid	0.3	0.8	None	None	Dark brown	Brown
trans-ρ-coumaric acid	0.5	0.5	Light blue	deep blue	Light brown	Blue
Vanillic acid	0.8	0.6	None	None	Orange	Purple
P–coumarylquinic acid	1	0.6	None	None	Light yellow	Yellow
Cis-ρ-coumaric acid	0.4	0.8	Light blue	deep blue	Dark brown	Blue
Meliotic acid	0.5	0.8	None	None	Orange yellow	Purple
Cis-Ferulic acid	0.9	0.7	Light blue	Blue	Purple	Blue green
trans-Ferulic Acid	0.8	0.3	Light blue	Blue	Purple	Blue green
Coumarin	1	0.7	None	None	Yellow	Violet
Salycyclic acid	0.9	0.3	Dark blue	Dark violet	Yellow	Pink
Cinnamic acid	1	0	None	None	Yellow	Green
Syringic acid	0.9	0.5	None	None	red	Dark blue

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Solvents:

1. Benzene:Acetic acid : Water → 60:70:30
2. Sodium Formate: Formic acid:Water → 10 :1 :200

B. Flavonoids Compounds

a) Extractive procedure

The flavonoid compounds were extracted according to the method given by Markham (1982). About 5g of powdered plant material was extracted in two steps. First 18ml of Methanol and 2 ml of water (9:1) were added, shaken well and kept for one day at room temperature. The supernatant solution of the extract was taken and transferred to another test tube. Secondly Methanol and water of 5ml each (1:1) was added to the remaining residue, stirred well and the mixture left for 24 hours. The two extracts were combined, mixed well and filtered through cotton wool. Later the filtrate was evaporated to about 1/3 the original volume till most of the methanol was removed. The resultant aqueous extract was extracted with chloroform repeatedly for 3 times. The solvent extracted aqueous chloroform layer was evaporated to dryness under vaccum in a rotary evaporator. The dried residue was saturated with 1 ml of 95% alcohol and stored in a dark place at low temperature.

b) Identification

The extracts containing flavonoids were separated in suitable quantities on Whatmann No.1 Chromatographic filter paper(23x29 cm) adopting the ascending technique using the solvent system Iso-propyl alcohol : Ammonia (25%) : Water(8:1:1:v/v/v) and n-butanol:acetic acid: water(4:1:5v/v/v) (top layer was used).The dried Chromatograms were examined under UV lamp and the fluorescent spots were marked. The papers while exposed to Ammonia Vapours were also observed under UV lamp and the new fluorescent regions were marked. The flavonoid compounds were identified by comparing the R_f values and colours with those of the authentic samples on the Chromatograms. Co-Chromatographic studies were conducted with authentic markers to confirm the identification.

C) Chromogenic spray reagents

Diazotized sulphanilic acid reagent (Markham, 1982) :0.3% solution of sulphanilic acid in 8% HCl (25ml) was mixed with 5% Sodium nitrite solution(1.5ml) just before use. The Chromatographic sheets were sprayed with this mixture and with a 20% solution of anhydrous Sodium carbonate.

1% Alcoholic ferric chloride:1 g of Ferric chloride was dissolved in alcohol and made up to 100ml.

1% Alcoholic Aluminium chloride:1g of Aluminium chloride was dissolved in alcohol and made up to 100ml, just before spraying of the chromatographic sheets

Table: Flavonoids:

Compound	Stem Bark	Gum
Quercetin	+	+
Rutin	+	+
Myricetin	-	-
Luteolin	+	-
Apigenin	-	-
Orientin	+	-
Vitexin	+	+

Table: R_f values and colour reactions of Flavonoids on Paper Chromatograms:

Compound	R_f values in solvent		U.V Fluorescence		Sulphanilic Reagent	1% Alcoholic Ferric Chloride	1% Alcoholic Aluminium Chloride
	1	**2**	**Without NH3**	**With NH3**			
Quercetin	0.3	0.6	yellow	Light yellow	Bright yellow	Green	Yellow
Rutin	0	0.6	Orange brown	Yellow	Green	Olive	Grey yellow
Myricetin	0.1	0.4	Yellow	Bright yellow	Light yellow	Olive	Grey yellow
Luteolin	0.4	0.8	Dull yellow	Yellow	Light red	Pale green	Pale yellow
Apigenin	0.6	0.9	Red brown	Red brown	Pink	Pale green	None
Orientin	0.8	0.3	Yellow	Yellow green	Grey	None	None
Vitexin	0.9	0.4	Dull yellow	yellow	Bright red	none	None

Solvents:

1. Iso-propyl alcohol:Ammonia(25%) :Water → 8:1:1 & n-butanol:Acetic acid:Water → 4:1:5

C. Amino acids

a) Extractive procedure

Amino acids were extracted following the method of Das Chowdary *et al.,* (1967). About 5g of plant material was cut into small pieces and plunged immediately into a round bottomed flask containing 20ml of 80% Ethanol. It was reflexed for 30 minutes over a boiling water bath. The boiled material was ground in mortar using additional volumes of 10ml ethanol 80% (v) and centrifuged at 2000 rpm. The supernatant was collected and the Ethanol removed in vaccum. The aqueous extract was passed through Dowex 50W-X8 (H^+ from 20-50 mesh) column (1x10cm). the amino acids were eluted from the column with 50ml of 2N Ammonium hydroxide. The elute was evaporated to dryness and the residue was saturated with 95% Ethanol(1ml).

b) Identification

The extracts containing amino acids were seperated on Whatmann No.1 Chromatographic filter paper(23x28 cm) employing two-dimensional ascending technique using the solvent system:

1) Sec. butanol : Formic acid : Water(75:13:12v/v/v)-1st direction.
2) Buffer saturated phenol (1:2v/v)-2nd direction(6.3 g of Sodium citrate and 3.7g of Potassium dihydrogen phosphate were dissolved in 100ml of distilled water. To this 200ml of distilled phenol was added)

The amino acids were identified based on comparision of R_f values, colours and with those of authentic samples by co-chromatographic studies.

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C) Chromogenic spray reagents:

Ninhydrin: It was prepared by dissolving 200 mg of Ninhydrin in 98ml of n-butanol with 2ml of glacial acetic acid

Table: Amino acids:

Compound	Stem Bark	Gum
Aspartic acid	+	-
Arginine	+	-
Asparagine	+	+
α-Alanine	+	+
β-Alanine	+	+
2-Aminobutyric acid	-	+
Cystaine	+	+
Cystine	+	+
Glutamic acid	+	-
Glutamine	+	-
Glycine	+	-
Histidine	+	+
Isoleucine	+	-
Leucine	+	+
Lycine	+	+
γ-Methylene glutamic acid	-	-
γ-Methylene glutamine	-	+
Norleucine	-	+
Ornithine	+	-
Phenylalanine	-	-
Proline	+	+
Serine	+	+
Threonine	+	+
Tryptophan	-	-
Valine	+	+
Tyrosine	-	-

Table: R_f values and colour reactions of Amino acids on Paper Chromatograms:

Compound	R_f values in solvent		Colour with Ninhydrin
L-Aspartic acid	0.05	0.10	Light violet
DL-Arginine	0.10	0.60	Violet
DLAsparagine	0.19	0.41	Violet
α-Alanine	0.26	0.28	Light violet
β-Alanine	0.42	0.77	Deep violet
DL-2-Aminobutyric acid	0.55	0.70	Light violet
L-Cystaine	0.38	0.61	Deep violet
L-Cystine	0.23	0.53	Violet
L-Glutamic acid	0.17	0.20	Violet
L-Glutamine	0.14	0.82	Violet
L-Glycine	0.09	0.38	Violet
L-Histidine	0.08	0.52	Violet
DL-Isoleucine	0.68	0.82	Violet
L-Leucine	0.79	0.83	Deep violet
L-Lycine	0.79	0.83	Deep violet
γ-Methylene glutamic acid	0.20	0.68	Violet
γ-Methylene glutamine	0.30	0.71	Violet
DL-Norleucine	0.96	0.68	Violet
DL-Ornithine	0.04	0.27	Light violet
DL-Phenylalanine	0.60	0.90	Light violet
L-Proline	0.39	0.89	Yellow
DL-Serine	0.20	0.23	Deep violet
DL-Threonine	0.28	0.35	Deep violet
DL-Tryptophan	0.50	0.76	Violet
DL-Valine	0.59	0.77	Deep violet
L-tyrosine	0.52	0.65	Light violet

Solvents:

1. Sec. butanol : Formic acid :Water → 75:13:12
2. Buffer:Phenol → 1:2

D. Anthocyanidins:

a) Extractive procedure: The anthocyanidin constituents were extracted according to the method described by Harborne (1973). About 5g of plant material was immersed in 20ml of 2N HCl in a boiling test tube and heated for 30-40 minutes at6 100^{0}c. The extract was cooled and filtered. The filtrate was washed twice with 20 ml of ethyl acetate to remove flavones. The remaining aqueous extract was further heated at 80^{0}c for 3 minutes to remove the last traces of ethyl acetate. The pigment was re-extracted again with a small volume of Iso-amyl alcohol. The Ethyl acetate extract was evaporated to dryness on a boiling water bath. The anthocyanid in the residue was diluted with 1ml of 1% Methanolic HCl and preserved at low temperature in dark for future use.

b) Identification:

The extracts containing anthocyanidins was separated on 23 x 29 cm Whatmann No.1 Chromatographic filter paper. Unidimensional Chromatographic ascending technique was employed with Conc. Hydrochloric acid, Formic acid, Water (2:5:3 v/w/v) and n-butanol: Acetic acid : water(4:1:5). The Chromatograms developed from the said solvent systems were taken out from the glass chambers and dried at room temperature. The fluorescent regions of dried papers were marked under UV light. The R_f values and colours of the spots were determined by comparing with those of the authentic markers on the Chromatograms.

Table 4a:

Compound	Stem Bark	Gum
Cyanidin	+	-
Petunidin	+	-
Delphinidin	-	-

Anthocyanidins:

Table: R_f Values and Colours of Anthocyanidins on Paper Chromatograms:

Compound	R_f values in solvent		Visible Colour	U.V. fluorescence	
	1	**2**		**without NH_3**	**with NH_3**
1	0.4	0.7	Meganta	pink	blue green
2	0.3	0.5	Purple	mauve	Mauve
3	0.2	0.4	Purple	mauve	blue green

Solvents:

1) Conc. Hydrochloric Acid : Formic Acid:Water → 2:5:3
2) n-butanol : Acetic Acid : Water → 4:1:5

Thin layer Chromatography (TLC):

E. Lipids:

a) Extractive procedure:

Lipids were extracted following the method adopted by Hoppe and Heitefuss (1974). A solvent mixture consisting of 30ml Chloroform, 60ml of Methanol and 20 ml Water(1:2:0.8 v/v/v) was taken and allowed to boil. About 5g of plant material was homogenized in the above solvent mixture. The contents were filtered and taken separately. The residual mixture was treated with 70ml Methanol and

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the filtrate taken. The residue was again washed with 100ml Chloroform. All the above filtrate mixtures were taken in a separating funnel and 90ml of water added. The mixture now contains Chloroform**:** Methanol**:** Water in the ratio of 2**:**2**:**1.8 respectively and the mixture were allowed to settle. The lower lipid layer containing chloroform was separated and transferred into a breaker. The upper water layer was treated with 50ml of Chloroform successively for three times. All the extracted Chloroform layers were evaporated to dryness in a vacuum by rotary evaporation maintained at 40^0C. The residue was treated with 2ml of Benzene to remove the traces of chlorophyll if present. Now the final lipid residue was dissolved in 2 ml of Chloroform and stored at low temperature in dark until use.

b) Identification

Thin layer plates were prepared by spreading a slurry of silicagel-G (50g in 100ml distilled water) to 105 mm thickness over thin glass plates. The glass plates were air dried and stored at room temperature. Before using, the plates were heated at 110^0c for 30 minutes in a hot air oven for activation. Using micropipette, one gram equivalent of lipid extract was taken and potted on TLC plates. The spotted areas were allowed for immediate dryness with the help of a drier. The dried plates were run in uni dimensional ascending Chromatography by using TLC glass chambers. The chambers were saturated with developing solvents one day before the plates were developed.

Solvent systems:

1) Chloroform**:** Methanol**:** Acetic acid**:** Water (170**:**25**:**25**:**3 v/v/v/v)
2) Acetone**:** Benzene**:** Water(91**:**30**:**8 v/v/v).

The plates were placed in airtight tanks. The developed plates were removed and dried at room temperature and exposed to Iodine vapours to visualize all the lipid compounds.
The TLC plates were sprayed with 25% Sulphuric acid reagent with the help of an automizer for the clear detection of various lipid layers. The lipids were identified by comparision of R_f values, color and with those of authentic samples by Co-Chromatographic studies.

c) Chromogenic spray reagent:

The TLC plates were sprayed with 25% Sulphuric acid and heated to 230^0c for 15 minutes, and the colour observed. Glycolipids gave red brown while phospholipids gave bright, red and other lipids gave pale brown colour spots on white background

Table 5a: Lipids

Compound	Stem Bark	Gum
Phosphatidyl serine	+	+
Phosphatidyl inositol	+	-
Phosphatidyl Clioline	-	-
Phosphatidyl Ethanolamine	-	-
Digalactosyl Diglyceride	+	-
Phosphatidyl glycerol	+	+
Sulphoquinovosyl Diglyceride	+	+
Monogalactosyl Diglyceride	+	+
Steryl glycoside	+	+

Table 5b: Rf values and colour reaction of lipids on thin layer Chromatograms

Compound	R_f values in solvent	Colour with iodine vapours	Colour with H_2SO_4(25%)
1	0.09	+	+
2	0.13	+	+
3	0.18	+	+
4	0.23	+	+
5	0.33	+	+
6	0.41	+	+
7	0.57	+	+
8	0.81	+	+
9	0.87	+	+

Solvent :
$CHCl_3$: MeOH:HOAc :H_2O → 170:25:25:3

5. Determination of ash values

The object of ashing crude drugs is to remove all traces of organic matter which may otherwise interfere in an analytical determination. The inorganic salts naturally occurring in drug or adhering to it or deliberately added to as a form of adulteration are removed by gradual incineration of the drug. Thus the residue remaining after incineration of the powdered drug is known as ash content. The ash values are a criterion to judge the identity or purity and quality of the crude drug.

a) Determination of total ash:

Total ash usually consists of Phosphates, Silicates and Carbonates of Potassium, Calcium and Magnesium. About 2 gm of the air dried powdered drug was accurately weighed and taken in a tared silica crucible, which was previously ignited and weighed. The powdered drug was spread as a fine even layer at the bottom of the crucible. The crucible containing the drug was gradually incinerated in a muffle furnace by increasing the temperature. It was heat dull red hot until free from carbon or converted into ash. Later the crucible was allowed to cool and kept in desiccators. The residue was weighed the percentage of total ash calculated with reference to the air dried sample. The residue was weighed the percentage of total ash calculated with reference to the air dried sample. The procedure was repeated till constant result was obtained.

b) Determination of acid insoluble ash:

Acid insoluble ash is a part of total ash insoluble in dilute Hydrochloric acid. The acid insoluble ash content is determined and recommended for certain drugs may be coated with dirt and sand. The total ash obtained as described above was treated with 25ml of 10% Hydrochloric acid and boiled for 5 minutes. The insoluble matter was filtered and collected on ashless filter paper (Whatman No.42) and the paper washed with hot water. The insoluble ash was ignited and weighed in a tared silica crucible. The acid insoluble ash of the drug was repeated to get constant results.

c) Determination of water soluble ash:

The total ash obtained from the above process was boiled with 25 ml of distilled water for 5 minutes. The insoluble ash was filtered through ash less filter paper (Whatman

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No.42). The residue was once again washed with water and ignited up to temperature not exceeding 450°c and converted into ash. The difference between the weight of total ash and insoluble matter represent the weight of water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried drug.

B. Determination of extractive values:
Extractive values indicate the nature of the constituents present in a crude drug. Based on the diversity in chemical nature and properties of the contents of the drug, various solvents were used for the determination of extractives. The solvents used for extraction dissolve appreciable quantity of substances desired. The methods used to find out the extractive values were as follows.

a) Determination of alcohol soluble extract:
Alcohol is an ideal solvent frequently employed to determine various chemical contents like Tannins, Resins etc. Generally, Ethyl alcohol is used for determination of alcohol-soluble extractive. Dilute Alcohol a may also be used in some cases, depending upon solubility of constituents in a crude drug. In the present work, the author used 100 ml of 90% Alcohol for determining crude drug was macerated with 100 ml of 90% Alcohol in a stoppered flask for 24 hours. It was shaken frequently every 6 hours and allowed to stand to 18 hours. Then the extract was filtered rapidly through filter paper, taking precaution against loss of alcohol, about 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish, transferred to the oven and dried at 105°C and weighed. The percentage of alcohol soluble extract was calculated with reference to the air dried drug.

Table: Ash value of various parts *Boswellia ovalifoliolata*

S.No.	Part(s)	Total Ash (%)	Acid insoluble ash (%)	Water insoluble ash (%)
1	Stem bark	13.5	2.50	2.00
	Gum	6.00	1.60	1.50

Table: Extractive value of the various parts *Boswellia Ovalifoliolata*

S.No.	Part(s)	90% alcohol Soluble Extract (%w/w)	Water Soluble Extract (%w/w)	Chloroform Soluble Extract (%w/w)	Pet.Ether Soluble Extract (%w/w)
1	Stem bark	17.6	22.4	4.8	3.4
	Gum	10.5	9.2	1.5	1.3

6. Fluorescence Studies

Introduction:
When physical and extractive value methods are inapplicable, especially powdered drugs, the fluorescence studies method will be useful to identify the drug. The study of fluorescence of powdered drugs as a means of identification appears to possess distinct possibilities of practical application (Chaudhuri Rai 1969). The ultra-violet light (200-400mu) is very active where several crude drugs show characteristic useful for their evaluation. Goodwin (1953) presented an excellent survey of fluorescent compounds in plants and the application of fluorimetry to their assay.

Fluorescence analysis was carried out following Chase and Pratt (1949) and Kokoski *et al.* (1958). The selected powdered drugs were studies both in Visible light and Ultra-Violet light for their fluorescence characters.

About one gram of the dried plant powder was extracted with 10 ml of the absolute alcohol. The tube containing Alcoholic extract of plant powder was placed at a distance of 14 cm from the source of Ultra-Violet tube and fluorescence observed. 2 ml of Alcoholic extract was treated with following reagents with the help of 1 ml pipette with 10 divisions.
Reagent I - Saturated Aqueous solution of $AgNO_3$
Reagent II - 0.1N Aqueous solution of NaOH
Reagent III - 5% Aqueous solution of HgCl2
The following tests were applied for fluorescence study

Test 1
a) Add 1 drop of Reagent I to 2ml of Alcoholic extract. It was shaken and fluorescence was observed.
b) 3 drops of Reagent II were added to the above (i.e.,a) and fluorescence observed immediately.

Test 2
a) Add 2 drops of Reagent II to 2 ml of Alcoholic extract, shaken and the colour of fluorescence was observed.
b) One drop of reagent III was added to the above containing extract (i.e.,a) shaken and noted the colour of fluorescence.
c) One more drop of Reagent III was added to the above (i.e.,a) shaken and noted the colour of fluorescence.
d) To the above (i.e.,c) 2 more drops of Reagent III were added, shaken and observed colour of fluorescence.

Dry powder also studies with the specified reagents. To the dry powder, a few drops of the following reagents were added separately and fluorescence observed immediately under Ultra-Violet tube light.
1) Powder as such 2) In NaOH in methanol 3) In NaOH in water 4) In HCl 5) 50% HNO_3 6) 50% H_2SO_4 P
2) **Table: Fluorescence analysis of the Alcoholic extract of the selected powdered drugs of *Boswellia ovalifoliolata*:**

Treatment of Powder	Stem bark	Gum
Colour of the extract in visible light	D. r	P. y
Colour of the extract under UV light	Y	B
Test - I		
Step 1 Reagent I(drop)	Pu	L. b
Step 2 Reagent II(3drops)	D.br	B
Test - II		
Step 1 Reagent II(2 drops)	Fl. pu	B
Step 2 Reagent III(1 drop)	De. pu	L. b
Step 3 Reagent III(1 drop)	G	L. b
Step 4 Reagent III(2 drops)	D. g	B

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Table: Fluorescence analysis of the dry powder of selected drugs with various reagents under U.V. light

Treatment of powder	Stem Bark	Gum
Powder in visible light	D. br	P. y
Powder in U.V light	D. pu	L. b
Powder + NaOH in Methanol	Fl. p	L. g
Powder + HCl	V	B
Powder + 50% HNO_3	G. P	Pu
Powder + 50% H_2SO_4	Pu	Brt.b

B = Blue ; Bl. r = Blood red; Br. g = Brownish green; Brt. b = Bright blue; Brt. fl. g = Bright fluorescent green; D.b = Dark blue; D.br = Dark brown; D.g = Dark green; D.p = Dark pink; D.pu = Dark purple; D.r = Dark red; D.y = Dark yellow; De.g = Deep green; De.pu = Deep purple; De.v = Deep violet; D. y. brn = Dark Yellowish Brown; Fl. b = fluorescence blue; Fl. g = Fluorescence green; Fl. p. g = Fluorescence pale green; Fl. pu = Fluorescence purple.

G = Green; G.br = Greenish brown; G. pu = Greenish purple; G. o = Greenish orange; G. v = Greenish violet; G. y = Greenish yellow; H. Fl. g = High green fluorescence. I. fl. g = Intensified green fluorescence.L. b = Light blue; L.br = Light brown; L. g = Light green, L.y.br = Light yellowish brown; O. r = Orange red; Ol. g = Olive green; P = pink; Pu. g = Purplish green; P. y = Pale yellow; R=Red; R. y = Reddish yellow. V= Violet; Y= Yellow; Y.br = Yellowish brown; Y. g =Yellowish green; Y. r = Yellowish red.

Table: Histochemical tests for stem bark of *Boswellia ovalifolilata:*

Drug	Reagent	Test for	Reaction	Result
Section	Iodine solution	Starch	Blue colour	+
Section	10% Ferric chloride solution + alcohol	Tannins	Black colour	+
Section	Sudan III solution	Oil globules	No change	-
Section	Conc.Hcl	Crystals	Effervescence	+
Section	Pinch of Phloroglucinol +dil.Hcl + Alcohol	Lignin	Magenta colour (Fibres)	+

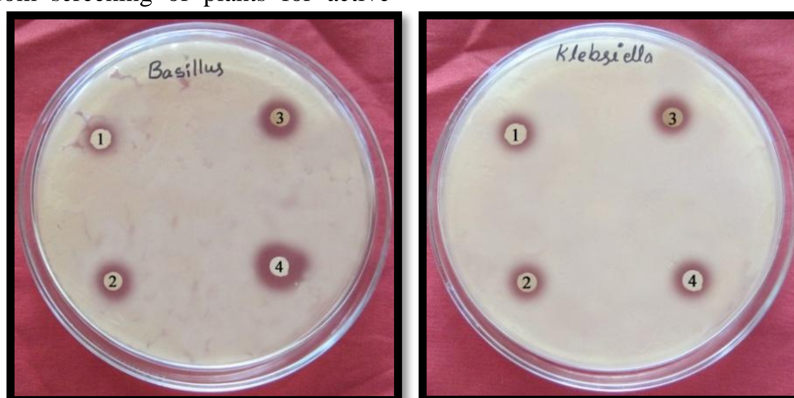
Antibacterial Activity

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. The presence of antimicrobial substances in the higher plants provide a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. The antimicrobial compounds from plants may inhibit microbial growth by different mechanism than those presently used antimicrobials and may have a significant clinical value in treatment of resistant microbial strains. Therefore, random screening of plants for active chemicals is as important as the screening of ethnobotanically targeted species (Principle, 1989).

Bacterial Cultures:

The bacterial cultures were procured from the Department of Microbiology, Sri Venkateswara Institute of medical Sciences (SVIMS), Tirupati, Andhra Pradesh. Pathogenic Gram positive bacteria such as *Bacillus subtilis*, *Staphylococcus aureus*, and Gram negative bacteria *Proteus vulgaris*, *Pseudmonas aeruginosa,* were maintained on nutrient agar slants at 4^0C until further used for experimental studies.

Antibacterial activity of *Boswellia ovalifoliolata*:



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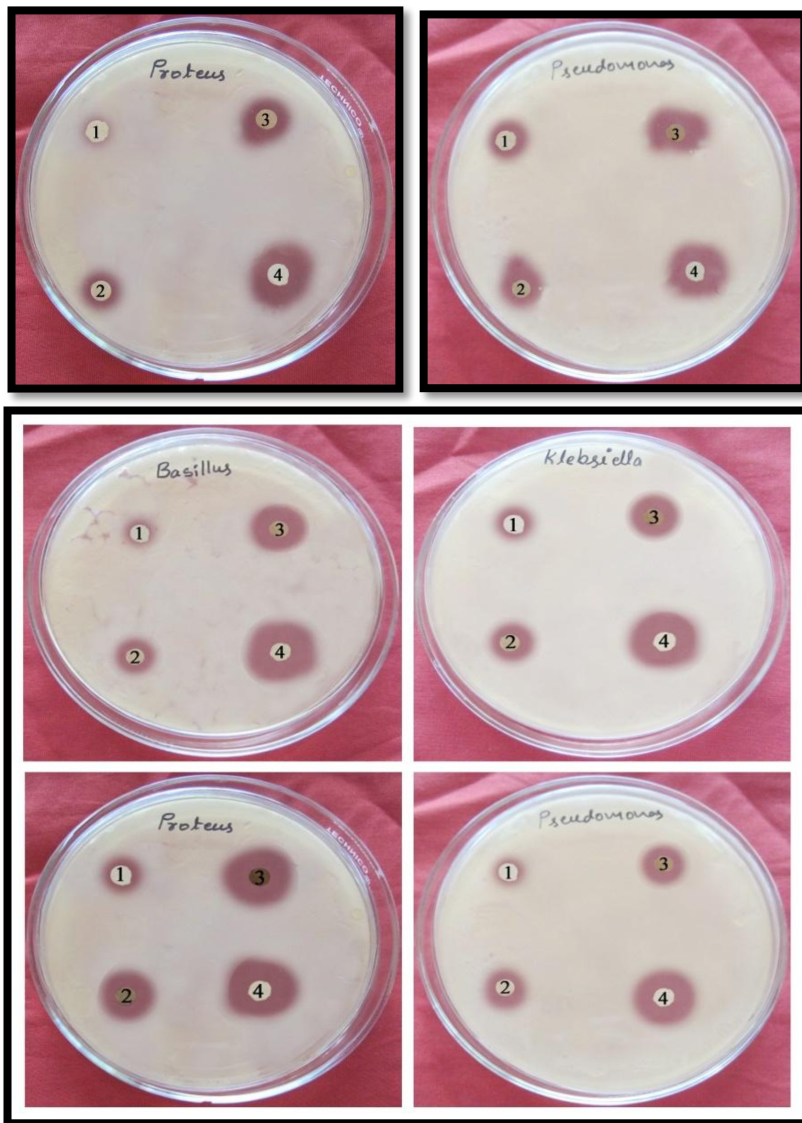
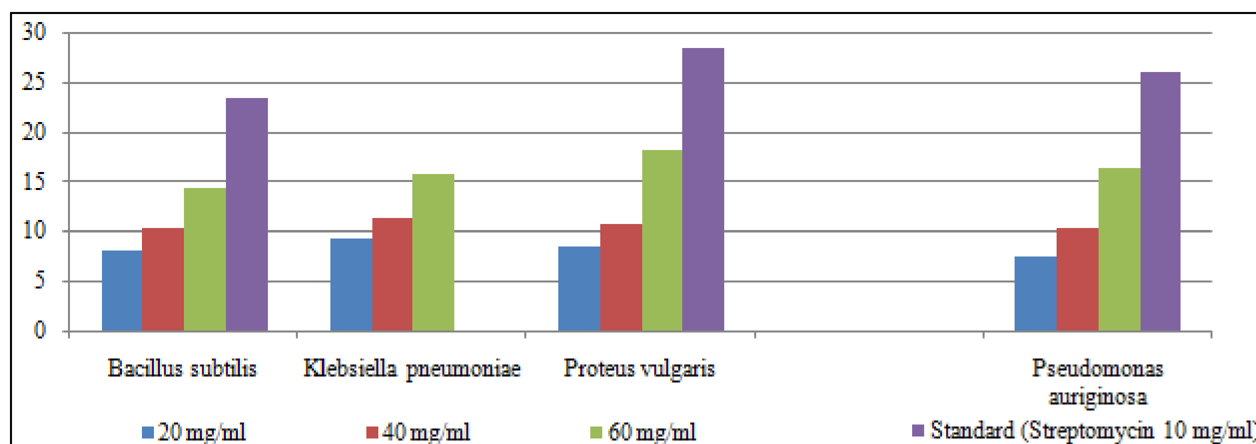


S. No	Name of the microorganism	20 mg/ml	40 mg/ml	60 mg/ml	Standard (Streptomycin 10 mg/ml)
1	Bacillus subtilis	8.14± 0.24mm	10.32 ±0.61mm	14.35 ± 0.34mm	23.45 ± 0.65mm
2	Klebsiella pneumonia	9.32 ± 0.56mm	11.45 ± 0.32mm	15.8 ± 0.71mm	24. 4 ± 0.53mm
3	Proteus vulgaris	8.43 ± 0.64	10.7 ± 0.89mm	18.24 ± 0.46mm	28.53 ± 0.74mm
4	Pseudomonas auriginosa	7.54 ± 0.23 mm	10.3 ± 0.45mm	16.32 ± 0.29mm	26.25 ± 0.36 mm



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7. Results

Present investigation, anti bacterial activity of four selected medicinal plant, on pathogenic bacterial strains, all exhibited significant antibacterial activity. Methanol stem extract of *Boswellia ovalifoliolata* at the concentration 60 mg/ml showed potent antibacterial activity. *P. vulgaris* (18mm) was more susceptible, followed by *P. aeruginosa* (16mm), *K. Pneumonia* (15mm) and *B. subtilis* (14mm).

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