

EVALUATION OF ANTIMICROBIAL ACTIVITY OF LITSEA GLUTINOSA

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Abstract

Litsea glutinosa is widely available throughout India. Litsea glutinosa is called as Indian Laurel in English and as Medhasaka in Sanskrit belonging to the family Lauraceae. The powdered material of Litsea glutinosa bark was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from Petroleum ether, Ethanol, to finally water. After extraction the extracts were subjected to Lyophilization to get dry extract & preserved in aseptic condition. The dried extracts were subjected to various phytochemical analysis to detect the presence of different phytoconstituents like alkaloids, glycosides, flavonoids, saponins, tannins, phenolic compounds. The petroleum ether extract, ethanolic extract and aqueous extracts of the Litsea glutinosa bark have the antibacterial & antifungal activity against Gram positive Staphylococcus aureus bacteria using reference standard like Procain pencillin, Gram negative like Pseudomonas aeruginosa, Salmonella typhi, E-coli bacteria using reference standard like Streptomycine sulphate. And fungal species like Aspergillus fumigatus and Candida albicans using reference standard like Griseofulvin. The bark of Litsea glutinosa, “is one of the most popular of native drugs”, is considered to be capable of relieving pain, arousing sexual power & good for stomach are considered to be mildly astringent, include treatment of diarrhoea & dysentery. Litsea glutinosa is widely used as a demulcent & as an emollient. Petroleum ether extract & Ethanolic extracts showed good activity against Pseudomonas aeruginosa. Aqueous extract & Petroleum ether extracts showed less activity as that of Ethanolic extract against Salmonella typhi. Then Ethanolic extract showed more effective activity against E-coli. Ethanolic extract had very high activity against Staphylococcus aureus. Likewise Petroleum ether extract of Litsea glutinosa effective in inhibiting the growth of Aspergillus fumigatus & Candida albicans. The phytochemical constituents of bark of Litsea glutinosa showed the effective antibacterial & antifungal activity.

Key words : Litsea glutinosa, Bark extracts, Antibacterial activity, Antifungal activity, Phytochemicals, Pathogens.

Introduction

Traditional knowledge regarding of medicinal plants and their use by indigenous cultures are not only useful for conservation of cultural traditions and biodiversity but also for community healthcare and drug development in the present and future. Due to the poor sanitary conditions, Sunburns Psoriasis and to the climatic conditions the infections of the skin are common in the rural areas. Therapy with synthetic tropical applications have most side effects and cannot be afford by the people due to import

cost of the drug, to overcome this problem plants growing around us are utilized without scientific validation.

The use of higher plants and their extracts to treat infections is an age-old practice. Traditional medicinal practice has been known for centuries in many parts of the world. Ayurveda, the science of life, prevention and longevity is the oldest and most holistic medical system available on the

planet today. Herbal medicines are gaining interest because of their cost effective and eco-friendly attributes^[3].

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization^[4], 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. Therefore, such plants should be investigated to better understand their properties, safety and efficiency^[1].

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency^{[6][8][12][2][14][11][10]}. Many plants have been used because of their antimicrobial properties, which are due to compounds synthesized in the secondary metabolism of plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils^[5], as well as in tannin^[13].

Litsea glutinosa is widely available throughout India. The fresh dried stem barks were collected from the forests of Ananthanahalli near Harapanahalli, Karnataka. The bark was subjected to coarse powdered (#:44) to obtain uniform texture. The sieved powder was stored in air tight & high density polyethylene containers before extraction. The powdered material of *Litsea glutinosa* bark was extracted separately by continuous hot extraction process using soxhlet apparatus with different solvents in increasing order of polarity from petroleum ether, ethanol & aqueous extracts were subjected to lyophilization to get dry extract & preserved in aseptic conditions. The dried extracts reconstituted in DMSO.

So we have used the Petroleum ether, Ethanolic, Aqueous extracts of the *Litsea glutinosa* bark, these all extracts have antibacterial and antifungal activity against gram-positive *S.aureus* bacteria using reference standard like Procaine penicillin, gram-negative like *P.aeruginosa*, *Salmonella thphi*, *E-coli* bacteria using reference standard like Streptomycine sulphate and fungal species *Aspergillus fumigatus*. *Candida albicans* using reference standard like Griseofulvin.

MATERIALS AND METHODS :

I. Preliminary phytochemical screening:

Phytochemistry is the chemistry dealing with plants or plant products or natural products. The natural products comprise different therapeutically active (alkaloids, glycosides, flavonoids etc.) or inactive (starch, cellulose etc.) chemical constituents. The extract was subjected to preliminary qualitative chemical tests^{[7][9]}.

Carbohydrates:

Carbohydrates are the most abundant class of organic compounds found in living organisms. Chemically, carbohydrates are simple organic compounds that are aldehydes or ketones with many hydroxyl groups added and composed of carbon, hydrogen and oxygen. The basic carbohydrate units are called monosaccharides and general stoichiometric formula of an unmodified monosaccharide is $(CH_2O)_n$ i.e. hydrates of carbon. Carbohydrates are mainly grouped in to two major classes as simple sugars (saccharides) and polysaccharides^{[7][9]}.

Chemical tests:

1. Molisch's Test (General test): The test is positive for soluble as well as insoluble carbohydrates. To 2-3 ml of aqueous extract add few drops of Molisch's reagent (alpha-naphthol in alcohol) and concentrated sulphuric acid along sides of test tube – Violet colour ring at the junction of two liquids.
2. Test for reducing sugars
 - i. Fehling's Test: Mix 1ml of T.S.+ 1ml of Fehling's solution A + 1ml of Fehling's solution B and boil on water bath for 5-

- 10 min – First a yellow, then brick red ppt is observed.
- ii. Benedict's Test: Mix equal volume of Benedict's reagent and test solution in test tubes and heat on boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.
 3. Test for monosaccharides
 - i. Barfoed's Test: Mix equal volume of Barfoed's reagent and test solution. Heat on boiling water bath for 1-2 min and cool. Red colour ppt is observed.
 4. Test for non- reducing sugars
 - i. Test solution does not give response to Fehling's and Benedict's tests.

Alkaloids :

Alkaloids are heterogeneous group of natural substances which are basic in nature and contain one or more nitrogen atoms and possess specific physiological actions when used in small quantities. The qualitative chemical tests used for detection of alkaloids are depends on characteristics of alkaloids to give precipitates as salts of organic acids or with compounds of heavy metals like gold, platinum, mercury etc. the different reagents used are Mayer's reagent (potassium-mercuric-iodide solution), Dragendorff's reagent (potassium-bismuth-iodide solution), Hager's reagent (picric acid) and Wagner's reagent (iodine-potassium-iodide solution). Special tests are required for highly water soluble alkaloids like caffeine^{[7][9]}.

Chemical tests:

Evaporate the alcoholic extract and to the residue add dilute HCl. Shake well and filter, perform the following tests with filtrate.

1. Mayer's Test: 2-3 ml of filtrate + few drops of Mayer's reagent – Creamy white ppt.
2. Wagner's Test: 2-3 ml of filtrate + few drops of Wagner's reagent – Reddish brown colored ppt.
3. Dragendorff's Test: To 2-3 ml of filtrate add few drops of Dragendorff's reagent- Orange red colored ppt. were obtained.
4. Hager's Test: 2-3 ml of filtrate + few drops of Hager's reagent – Yellow colored ppt.

Glycosides:

Glycosides are the organic compounds from plants or animal sources which on enzymatic or acid hydrolysis gives one or more sugar moieties (glycone) along with non- sugar moieties (aglycone or genin). Chemically, they are the acetals or sugar ethers formed by interaction of hydroxyl group each of non-sugar and sugar moieties, with a loss of water molecule. The linkage between glycone and aglycone is called glycosidic linkage and on the basis of this linkage, alpha and beta stereoisomers are assigned^{[7][9]}.

Chemical tests:

1. Keller Killiani Test (for deoxysugars): To 2 ml of extract add few drops of glacial acetic acid, 1 drop of 5% FeCl₃ solution and conc. H₂SO₄. Reddish brown color appears at the junction of two liquid layers and upper layer appears bluish green.
2. Legal's Test (test for cardenoloids): To aqueous or alcoholic extract, add 1 ml of pyridine and 1ml of sodium nitroprusside solution. Pink to red color appears.
3. Modified Borntrager's Test (for C-glycosides): To 5 ml of extract, add 5 ml of 5% FeCl₃ and 5 ml dil. HCl. Heat for 5 min on boiling water bath. Cool and add benzene or any organic solvent, shake well. Separate organic layer, to which add equal volume of dilute ammonia. Ammonical layer shows pinkish red color.

Flavonoids:

The flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene rings joined by a linear three carbon chain and are categorized, according to chemical structure, in to flavonols, flavones, flavanones, isoflavones, catechins, anthocyanidins and chalcones. The term flavonoid (or bioflavonoid) refers to a class of plant secondary metabolites and are most commonly known for their antioxidant activity. Flavonoids are mostly yellow colored substances and chemically they are derivatives of phenyl benzo gamma-pyrone ring^{[7][9]}.

Chemical tests:

1. Shinoda Test (Mg/HCl): 2-3 ml of test solution + pinch of Mg powder + 2-3 drops of conc. HCl – Deep red or magna color.
2. Pew's Test (Zn/HCl): Pinch of Zn powder + few ml of conc. HCl + 1-2 ml of test solution, which gives deep purple red color.
3. Few ml of alcoholic extract + 1-2 drops of conc. H₂SO₄ + magnesium turnings- Deep cherry red color.
4. To 1 ml of aq NaOH add 1 ml of test solution → Yellow color, which decolorizes after addition of acid.
5. Addition of lead acetate solution to small quantities of residue gives yellow colored ppt.

Saponins:

Specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produced when shaken in aqueous solutions, and structurally by their being composed of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative^{[7][9]}.

1. Foam Test: Extract is shaken vigorously with distilled water in test tubes Honey comb like foam is produced.

Tannins:

Tannins are astringent, bitter plant polyphenols that either bind precipitate or shrink proteins. They form colloidal solutions with water and are non-crystalline substances.

Tannins exhibits some chemical reactions,

1. Gelatin Test: Extract is dissolved in 2% gelatin + 10% NaCl – White precipitate.
2. Extract + alcoholic vanillin solution + dilute HCl – Pink color.
3. Extract + Lime water – Brown color.
4. Tannins are precipitated by salts of copper, tin and lead.
5. Tannins show color reactions with iron salts. Ferric chloride gives bluish black or bluish green color and potassium ferricyanide with ammonia gives deep red color.

Phenolic compounds:

Phenolic compounds are plant-based materials, phytochemicals which are synthesized primarily from products of the shikimic acid pathway, have several important roles in plants. Tannins, lignans, flavonoids, and some simple phenolic compounds serve as defenses against herbivores and pathogens^{[7][9]}.

1. To 2 ml of test solution add 0.5 ml FeCl₃ solution – Formation of intense color.

II. Antibacterial activity:

The compounds were tested in-vitro for their antibacterial activity against four microorganisms viz. Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi which are pathogenic in human beings.

Method:

Cup-plate agar diffusion method using Nutrient agar.

Materials used :

- i. Nutrient broth (Himedia)
- ii. Nutrient agar (Himedia)
- iii. 18-24 hrs growth culture in nutrient agar
- iv. Sterile petridishes
- v. Sterile micropipettes
- vi. Sterile cotton swabs
- vii. Sterile cork borer
- viii. Sterile test tubes

Preparation of Nutrient broth:

- i. Nutrient broth - 3.8 gm
- ii. Distilled water - 100 ml

Above components were dissolved in 100 ml distilled water and pH was adjusted to 7.2. This solution was sterilized by autoclaving at 15 psi. for 20 min.

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^oC for 18 – 24 hrs.

Preparation of medium (Nutrient agar):

- i. Nutrient agar - 2.8 gm
- ii. Distilled water - 100 ml

The agar was dissolved in to distilled water and pH was adjusted to 7.4+0.2. It was sterilized by autoclaving at 15psi for 20 min.

Preparation of test solutions:

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 ml) to give stock solution of concentration 1000 mcg/ml. Then 0.1 ml of this solution was used for testing.

Preparation of standard solution:

Standard drug Procain pencillin and Streptomycin sulphate were used. The concentration was 100mcg/ml.

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 cork borer of 8 mm diameter.

Then the solutions of test compounds (0.1) were added in cups by using micropipettes and these plates were incubated at 37⁰C for 48 hrs. The zone of inhibition was measured in mm for each organism.

III. Antifungal activity :

The compounds were tested in-vitro for their antifungal activity against *Candida albicans* and *Aspergillus fumigatus*.

Method:

Cup-plate agar diffusion method using Sabouraud-Dextrose agar.

Materials used :

- i. Sabouraud-Dextrose agar
- ii. 18-24 hrs growth culture on Sabouraud-Dextrose agar
- iii. Sterile petridishes
- iv. Sterile micropipettes
- v. Sterile cotton swabs
- vi. Sterile cork borer (8 mm)
- vii. Sterile test tubes

Preparation of Sabouraud-Dextrose medium:

- i. Dextrose - 40 gm
- ii. Neopeptone - 10 gm

Above components were dissolved in distilled water (1000 ml) and pH was adjusted to 5.5 – 6.0. This solution was sterilized by autoclaving at 121⁰C for 10 min.

Preparation of Inoculums:

One day prior to these testing, inoculations of the above fungal cultures were made in the Sabouraud-Dextrose medium and then incubated at 37⁰C for 18 – 24 hrs.

Preparation of Sabouraud-Dextrose agar:

- i. Dextrose - 40 gm
- ii. Neopeptone - 10 gm
- iii. Agar - 15 gm

All these components were dissolved in distilled water (1000 ml) and pH was adjusted to 5.5 - 6.0. It was sterilized by autoclaving at 121⁰C for 10 min.

Preparation of test solutions:

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 ml) to give stock solution of concentration 1000 mcg/ml. Then 0.1 ml of this solution was used for testing.

Preparation of standard solution:

Standard drug Griseofulvin was used. The concentration was 100mcg/ml.

Method of testing:

Sabouraud-Dextrose 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 cork borer of 8 mm diameter, corresponding to control, standard and test solution.

The solutions of each test compounds (0.1) were added in cups by using micropipettes and these plates were incubated at 37⁰C for 48 hrs. The zone of inhibition was measured in mm for each organism.

RESULTS:

Phytochemical screening of Litsea glutinosa bark extract:

The *Litsea glutinosa* bark extract was subjected to preliminary qualitative chemical tests. These tests were conducted to know the constituents, present in the plant extract. The

Litsea glutinosa bark extract has showed the presence of alkaloids in Aqueous extract, Ethanolic extract, and Petroleum ether extract. Sterols were absent in all the three kinds of extracts. Glycosides & Carbohydrates were present in all the extract of plant. Mucilage was absent in Aqueous extract, Ethanolic extract as well as Petroleum ether extract. The presence of Starch was identified by iodine test-only the Aqueous extract has showed presence of starch but not the Ethanolic & Petroleum ether extract. Other test for the detection of starch was Tannic acid test, presence of tannic acid in all the extracts of plant has indicated the presence of starch. Proteins, Cardiac glycosides, Saponins, Tannins & Phenolics were present in all the three extracts of Litsea glutinosa, but the flavonoids were absent in the extracts. Finally the phytochemical screening of Litsea glutinosa bark extract constituents were Tannin, beta-sitosterols and Boldine, norboldine, laurotetanine, N-methylactinodaphnine, quercetine, sebiferine, litsiferine. Kaempferol-3-glucoside, aminoacids, quercetine-3-rhamnoside, Kaempferol-7-aminoglucoside, Pelargonidine-5-glucoside, naringenin-7-monorhamnoside, mono and sesquiterpines, beta-amirine acetate.

Sl. No	Constituents	Aqueous extract	Ethanolic extract	Petroleum ether extract
1	Alkoloides	+	+	+
2	Sterols	-	-	-
3	Glycosides	+	+	+
4	Carbohydrates	+	+	+
5	Mucilage	-	-	-
6	Starch			
	a. Iodine test	+	-	-
	b.Tannic acid test	+	+	+
7	Proteins	+	+	+
8	Carñac glycosides	+	+	+
9	Saponins	+	+	+
10	Tannins and phenolics	+	+	+
11	Flavonoides	-	-	-

Table 1 : Phytochemical screening of Litsea glutinosa bark extracts

- + → Presence of constituents
- → Absence of constituents

Antibacterial activity:

Petroleum ether extract, Ethanolic extract, aqueous extract of *Litsea glutinosa* has showed the antibacterial activity. *Pseudomonas aeruginosa* has showed more activity in the Petroleum ether extract & Ethanolic extract, least activity in aqueous extract. *Salmonella typhi* has showed good activity against Ehanolic extract, moderate activity in aqueous extract & poor activity against Petroleum ether extract. In E-coli significant activity was observed in Ehanolic extract, good activity against aqueous extract & least activity in Petroleum ether extract. DMSo was referred as blank showed minimum activity & Strptomycin sulphate as standard has maximum activity. Staphylococcus aerus, the gram+ve bacteria has showed maximum activity against Ethanolic extract, very good activity in Aqueous extract & has least activity in Petroleum ether extract. DMSo referred as blank has minimum activity & Procaine penicillin as standard has maximum activity. So all the extracts of *Litsea glutinosa* has antibacterial activity of both gram+ve and gram-ve bacteria.

Drugs	Diameter of mean zone of inhibition(mm) after 24 to 48 hours	
	<i>Pseudomonas aeruginosa</i>	
	50mg/ml	100mg/ml
Blank	16	16
Petroleum ether extract	17	19
Ethanolic extract	17	19
Aqueous extract	16	17
Streptomycin sulphate	29	29

Table 2: Effect of petroleum ether, ethanolic and aqueous extracts of Litsea glutinosa bark on Pseudomonas aeruginosa.

Drugs	Diameter of mean zone of inhibition(mm) after 24 to 48 hours	
	<i>Salmonella typhi</i>	
	50mg/ml	100mg/ml
Blank	12	12
Petroleum ether extract	11	12
Ethanollic extract	13	17
Aqueous extract	11	14
Streptomycin sulphate	24	24

Table 3: Effect of petroleum ether, ethanolic and aqueous extracts of Litsea glutinosa bark on *Salmonella typhi*.

Drugs	Diameter of mean zone of inhibition(mm) after 24 to 48 hours	
	<i>Escherichia coli</i>	
	50mg/ml	100mg/ml
Blank	13	13
Petroleum ether extract	14	17
Ethanollic extract	20	24
Aqueous extract	14	18
Streptomycin sulphate	30	30

Table 4: Effect of petroleum ether, ethanolic and aqueous extracts of Litsea glutinosa bark on *Escherichia coli*.

Drugs	Diameter of mean zone of inhibition(mm) after 24 to 48 hours	
	<i>Staphylococcus aureus</i>	
	50mg/ml	100mg/ml
Blank	13	13
Petroleum ether extract	13	15
Ethanollic extract	17	24
Aqueous extract	15	19
Procaine penicillin	28	28

Table 5: Effect of petroleum ether, ethanolic and aqueous extracts of Litsea glutinosa bark on *Staphylococcus aureus*.

Note :

1. Average of three plates have taken for each extract
2. Diameter of the cup = 8 mm
3. The values shown in parenthesis indicates activity index.
4. Activity index more than 0.5 is considered as significant activity.Hence Petroleum ether, Ethanolic extracts shows significant antimicrobial activity.

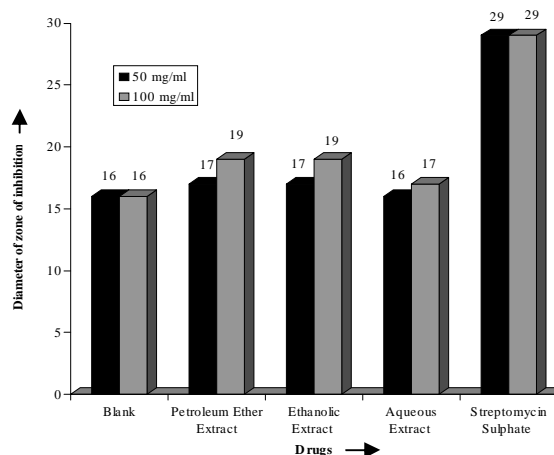


Fig.1 : Effect of petroleum ether, ethanol, aqueous extracts of Litsea glutinosa bark on *Pseudomonas aeruginosa*

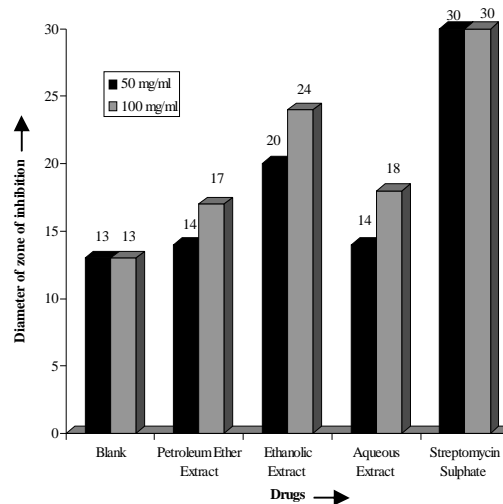


Fig.2 : Effect of petroleum ether, ethanol, aqueous extracts of Litsea glutinosa bark on *Escherichia coli*

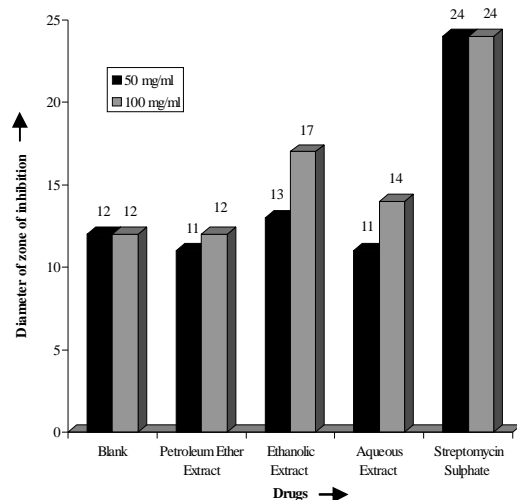


Fig.3 : Effect of petroleum ether, ethanol, aqueous extracts of Litsea glutinosa bark on *Salmonella typhi*

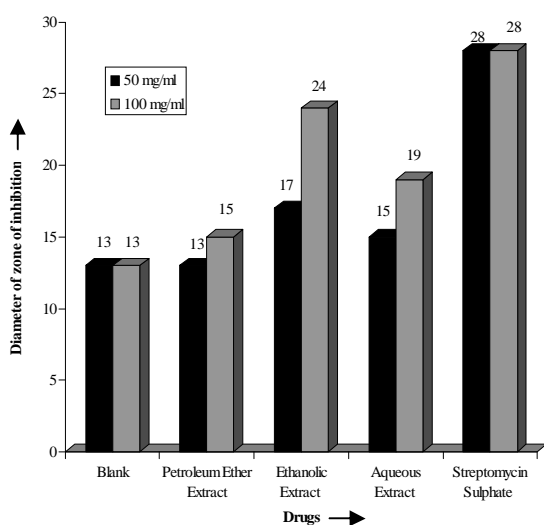


Fig.4 : Effect of petroleum ether, ethanol, aqueous extracts of Litsea glutinosa bark on *Staphylococcus aureus*

Antifungal activity:

Aspergillus fumigatus has showed the more activity against Petroleum ether extract, less activity in Ethanolic extract and poor activity in Aqueous extract. DMSo referred as blank & it has showed considerable activity. Griseofulvin as standard has showed average activity. Similarly *Candida albicans* also has the maximum activity against Petroleum ether extract and moderate activity in Ethanolic extract, least activity in aqueous extract. Here also DMSo referred as blank

with minimum activity & standard, Griseofulvin has activity within range. All the bacterial species have more activity against Ethanolic extract as compared to aqueous extract & Petroleum ether extract. Likewise all fungal species have showed effective activity against Petroleum ether extract, but not in the Ethanolic extract & Aqueous extract.

Drugs	Diameter of mean zone of inhibition(mm) after 24 to 48 hours	
	<i>Aspergillus fumigatus</i>	
	50mg/ml	100mg/ml
Blank	15	15
Petroleum ether extract	17	18
Ethanollic extract	13	14
Aqueous extract	13	14
Griseofulvin	17	17

Table 6: Effect of petroleum ether, ethanolic and aqueous extracts of Litsea glutinosa bark on *Aspergillus fumigatus*.

Drugs	Diameter of mean zone of inhibition(mm) after 24 to 48 hours	
	<i>Candida albicans</i>	
	50mg/ml	100mg/ml
Blank	14	14
Petroleum ether extract	19	23
Ethanollic extract	13	15
Aqueous extract	12	15
Griseofulvin	15	15

Table 7: Effect of petroleum ether, ethanolic and aqueous extracts of Litsea glutinosa bark on *Candida albicans*.

Note :

1. Average of three plates have taken for each extract
2. Diameter of the cup = 8 mm

- The values shown in parenthesis indicates activity index.
- Activity index more than 0.5 is considered as significant activity. Hence Petroleum ether, Ethanolic extracts shows significant antimicrobial activity.

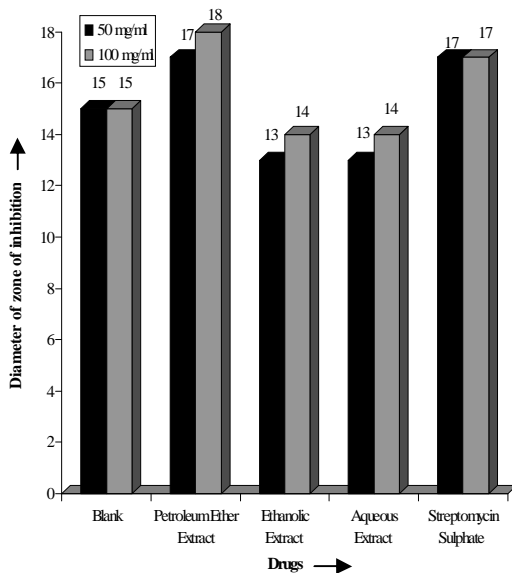


Fig.5 : Effect of petroleum ether, ethanol, aqueous extracts of Litsea glutinosa bark on Aspergillus fumigatus

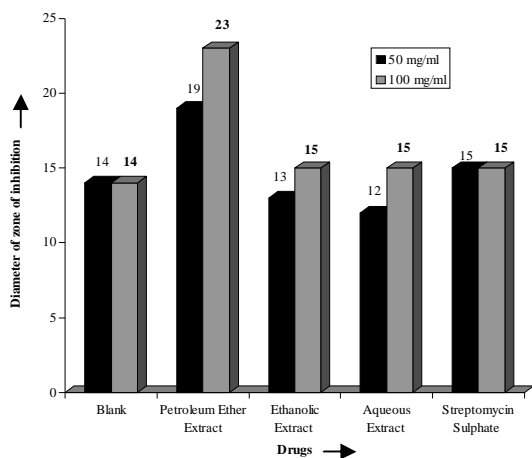


Fig.6 : Effect of petroleum ether, ethanol, aqueous extracts of Litsea glutinosa bark on Candida albicans

DISCUSSION:

Litsea glutinosa is one of the most popular of native drugs. The bark of *Litsea glutinosa* is widely used as a demulcent. Ground & pasted material is used as an emollient.

Mucilaginous bark is used in diarrhea & dysentery treatment and also for sprains, bruises & rheumatic gouty joints.

Petroleum ether & Ethanolic extract showed good activity as compare to the Aqueous extract against *Pseudomonas aeruginosa*. Aqueous extract & Petroleum ether extracts showed less activity as that of Ethanolic extract against *Salmonella typhi*. Then Ethanolic extract showed more effective activity against E-coli but the Aqueous extract & Petroleum ether extract showed moderate activity against E-coli. Ethanolic extract had very high activity, aqueous extract moderate & Petroleum ether extract showed less activity against *Staphylococcus aureus*.

So all extracts of *Litsea glutinosa* inhibited both gram-positive & gram-negative bacteria. Likewise Petroleum ether extract of *Litsea glutinosa* showed more effective activity against *Aspergillus fumigatus* & *Candida albicans* as that of Ethanolic & Aqueous extract of *Litsea glutinosa*. So the Petroleum ether extract of *Litsea glutinosa* inhibited the growth of fungal species.

Tannin, beta-sitosterols and Boldine, norboldine, laurotetanine, N-methylactinodaphnine, quercetine, sebiferine, litsiferine. Kaempferol-3-glucoside, aminoacids, quercetine-3-rhamnoside, Kaempferol-7-aminoglucoside, Pelargonidine-5-glucoside, naringenin-7-monorhamnoside, mono and sesquiterpines, beta-amirine acetate^[44]. These are the phytochemical constituents of bark of *Litsea glutinosa*, so it had showed the effective results of antibacterial & antifungal activity.

Conclusion

In this study *Litsea glutinosa* bark extracts showed the effective antimicrobial activity. After knowing the constituents of bark extracts, treated with bacterial & fungal species. *Pseudomonas aeruginosa* has showed more activity in Petroleum ether extract & Ethanolic extract, least activity in aqueous extract. *Salmonella typhi* has showed good activity against Ethanolic extract,

moderate activity in aqueous extract & poor activity against Petroleum ether extract. In E-coli significant activity was observed in Ethanolic extract, good activity against Aqueous extract & least activity in Petroleum ether extract. DMSO was referred as blank showed minimum activity & Strptomycin sulphate as standard has maximum activity. Staphylococcus aerus, the gram+ve bacteria has showed maximum activity against Ethanolic extract, very good activity in Aqueous extract & has least activity in Petroleum ether extract. DMSO referred as blank has minimum activity & Procaine penicillin as standard has maximum activity. *Aspergillus fumigatus* and *Candida albicans* both has showed the maximum activity against Petroleum ether extract & least activity in Ethanolic extract as well as Aqueous extract. Here also DMSO referred as blank showed considerable activity and Griseofulvin as standard has minimum activity. These results justify that, all the extracts of *Litsea glutinosa* has antibacterial activity of both gram+ve and gram-ve bacteria and also has effective antifungal activity.

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