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RESEARCH ARTICLE

DETERMINATION OF TOTAL PHENOLIC, FLAVONOID CONTENT AND ANTIMICROBIAL ACTIVITY

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Abstract

Plants are a rich source of phenolic compounds and one of the most important nature-based antioxidants. The compounds derived from plant-based extracts are an important pharmaceutical resource. This study was conducted to investigate the antioxidant activity and total phenolic and flavonoid content and to investigate the antibacterial effects of *Litsea glutinosa* bark extract. In this study, *Litsea glutinosa* was gathered and extracted by Soxhlet extraction using hydroalcoholic solvent. Total phenol content was measured by Folin-Ciocalcteu reagent and gallic acid standard, total flavonoid content was measured and antioxidant activity was investigated by DPPH with reference to ascorbic acid. Antimicrobial effects were investigated by disk diffusion with reference to ciprofloxacin. In this study, investigating the effects of different concentrations of hydroalcoholic *Litsea glutinosa* extract on pathogenic bacteria demonstrated that this extract exerted great inhibitory effects on both bacteria. The results indicated that the plants tested may be potential sources for isolation of natural antioxidant and antimicrobial compounds.

Keywords: Antioxidant activity, Total phenolic content, Flavonoid content, Antimicrobial effect, *Litsea glutinosa* (Lour).

INTRODUCTION

Litsea glutinosa (Lour.) C.B. Rob is an aromatic tree belongs to the family Lauraceae and is found to be sparsely distributed in the Western Ghats, India. *L. glutinosa* is an evergreen medium-sized tree and plant can attain a height of 20 meters.[1] *L. glutinosa* contain phytoconstituents like alkaloids, glycosides, flavonoids, saponins, tannins, phenolic compounds etc. The bark of *L. glutinosa*, "is one of the most popular of native drugs", is considered to be capable of relieving pain, arousing sexual power and good for the stomach in the treatment of diarrhea and dysentery. *L. glutinosa* is widely used as a demulcent and as an emollient. The phytochemical constituents of the bark of *L. glutinosa* have been shown to possess effective antibacterial and antifungal activity.[2] This species is critically endangered.[3] Sap of fresh bark or its decoction is prescribed as a remedy for diarrhea, dysentery, rheumatism, and as an aid to longevity. In addition, in current usage, a paste prepared by grinding bark with water is used as a plaster in cases of sprain, bruises, wounds, inflammation, back pain, rheumatic and gouty joints, bone fractures etc. It has analgesic, antiseptic and emollient effects.[4]

Some studies have indicated that plants are rich resources of antioxidant and antibacterial compounds and contain large amounts of secondary metabolites including phenolic compounds, flavonols, flavonoids, glycosides, and alkaloids.[5] Today, phythotherapy, as the use of plant-based products or herbal extracts, is a common approach worldwide. Given the side effects due to the use of chemical drugs and large costs spent for mass production of synthetic drugs, the secondary compounds of medicinal plants can be suitable alternatives to synthetic drugs.[6]

Since the antimicrobial activities of plants are partly attributed to the presence of secondary metabolites such as flavonoids, phenols, and antioxidants, and given the increasing use and efficacy of these compounds in treating diseases, it is highly important to investigate the extracts derived from plants, especially the plants traditionally used in medicine. In addition, the studies conducted on the medicinal plants gathered from different regions have reported widely inconsistent findings. This may indicate that the plants of different regions may exert different therapeutic effects.[7] This study was conducted to investigate the antioxidant activity, total phenolic and flavonoid content, and antimicrobial effects of *Litsea glutinosa*.

MATERIALS AND METHODS

Collection of plant

Litsea glutinosa plant barks were collected from the rural areas of Bhopal (M.P), India, in the month of December 2016.

Preparation of plant extract

Barks were collected in the shade dried and coarsely powdered after drying. The powdered material was subjected to extraction with petroleum ether by Soxhlet extraction procedure. The extraction was continued till the defatting of the material had taken place. After deffating, the plant material was subjected to hydroalcoholic extraction. The extract was filtered and it was finally dried at low room temperature under pressure in a rotary vacuum evaporator (Thermotech, Buchi type model th-012). The extracts were concentrated and stored in a well closed container.

Total phenolic content

The total phenolic content (TPC) of different extracts was estimated usingFolin–Ciocalteu (FC) method [8] with few modifications. The calibration curve was constructed with different concentrations of gallic acid (20-500 µg/ml) as the standard. Briefly, 1 ml of crude extract (1000 µg/ml) was added to 3 ml of distilled water. The sample was then mixed thoroughly with 1 ml of Folin–Ciocalteu reagent (Previously diluted 6 fold with distilled water (1:6, v/v), followed by the addition of 2 ml of 20% (w/v) sodium carbonate (Na2CO3). After 30 min of incubation at room temperature in the dark, the absorbance of the sample and the standard was measured at 765 nm. Distilled water was used as the reagent blank. The TPC of the sample was determined by using linear regression equation obtained from the calibration curve of gallic acid. The content of total phenolic compounds was expressed in milligrams (mg) of gallic acid equivalents (GAE) per gram (g) of the plant (dry extract).

Total flavonoid content

The total flavonoid content (TFC) of different extracts was determined using aluminium chloride colorimetric method [9] with slight modification. The calibration curve was plotted with different concentrations of quercetin (20-100 µg/ml) is used as a standard. In brief, 1 ml of plant extract (1000 µg/ml) was mixed with 0.1 ml of 10% aluminium chloride (AlCl3) solution, 0.1 ml of 1M potassium acetate (C2H3KO2) solution and 2.8 ml of double distilled water in a test tube. After 30 min of incubation at room temperature, the absorbance of the sample and

standard was measured at 415 nm with a Ultraviolet-visible spectrophotometer (Shimadzu, Japan). A solution containing all reagents except aluminium chloride, which is replaced by the same amount of distilled water, is used as a blank. The TFC of the sample was determined by using linear regression equation obtained from the calibration standard curve of quercetin. The content of total flavonoid compounds was expressed in milligram (mg) of quercetin equivalent (QE) per gram (g) of the plant (dry extract).

Antimicrobial activity

Pathogenic bacteria used

The pathogenic bacteria used in the current study are one gram negative and one gram positive obtained from Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India.

Method of preparation of media

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely.
Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch2 (121^{o}C) for 15 minutes.

Preparation of plates

After sterilization, the nutrient agar in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37^{o}C overnight to check the sterility of plates. The plates were dried at 50^{o}C for 30 minutes before use.

Revival of the bacterial cultures

The bacterial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient broth than incubated for 24 hours at 37^{o}C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the nutrient agar plates with loop full of bacteria and further incubated for next 24 hours at 37^{o}C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antimicrobial sensitivity

The antimicrobial sensitivity test is employed on to the all the bacteria used under present study with hydroalcoholic extract obtained from *Litsea glutinosa*. For this experiment 6 mm diameter well with stock of 100 mg/ml of extract separately then dried in aseptic conditions. A nutrient agar plate is seeded with particular bacteria with the help of spread plate technique prior and left for 5 minutes. Now culture plates then incubated for 24 hours at 37^{o}C. After incubation, plates were observed to see the sensitivity of extracts towards test bacteriums at particular concentration in the form zone of inhibition.

Antibiogram studies

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the 100 mg/ml concentration of phytoextract used in present study were prepared by transferring a loop of culture into sterile

nutrient broth and incubated at 37ºC for 24-48 hours. A loop full was taken from these broths and seeded onto sterile nutrient agar plates through sterile cotton swab to develop diffused heavy lawn culture.

The well diffusion method was used to determine the antibacterial activity of the extracts prepared from the *Litsea glutinosa* bark using standard procedure. There were 3 concentration used which are 25, 50 and 100 mg/ml for antibiogram studies. The plates were incubated at 37ºC for 24 hour and then examined for clear zones of inhibition around the wells with particular concentration of drug.[11]

Results and discussion

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **Y =** 0.008x + 0.009, R^2= 0.999, where × is the absorbance and y is the gallic acid equivalent (GAE).

Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve: Y=0.008 X+0.007, R^2=0.999, where X is the absorbance and Y is the quercetin equivalent (QE). The results are given in Table.

The total phenolic content in hydroalcoholic extract was 17.43 mg of gallic acid equivalent/g of dry extract and total flavonoid content was 60.15 mg of quercetin equivalent/g of dry extract. The results are given in Table 1.

Table 1: Results of total phenolic and flavonoid content

S. No.	Extracts	Total Phenol (mg/gm)	Total Flavanoid (mg/gm)
1.	Hydroalcoholic	17.43	60.15

Two microbes *S. Mutans* and *klebsiella pneumonia*were found to be sensitive to hydro alcoholic extracts of *Litsea glutinosa* at the initial test concentration of 100 mg/ml using well diffusion method. Further the results antibiogram studies at the 100, 50 and 25 mg per ml concentration of *Litseaglutinosa* bark extract on to the test organism using well diffusion method inhibitory activity are comparedwith the standard antibiotics Ciprofloxacinat the same concentration. (Table 3).

Table 3: Antimicrobial activity of standard drug

S. No.	Name of Drug	Microbes	Zone of inhibition		
			25mg/ml	**50mg/ml**	**100mg/ml**
1	Ciprofloxacin	*S. mutans*	15±0.15	20±0.13	25±0.19
		Klebsilla p.	15±0.09	18±0.12	27±0.08

Table 4: Antimicrobial activity of hydroalcoholic extract of *Litsea glutinosa*

S. No.	Microbes	Zone of inhibition		
		25mg/ml	50mg/ml	100mg/ml
1.	*S. mutans*	14±0.19	18±0.13	21±0.15
2.	*Klebsilla p.*	25±0.08	27±0.12	30±0.09

Figure 2: Antimicrobial activity of hydroalcoholic extract of *Litsea glutinosa*

Many studies have recently been conducted on the effects of different plant species on bacteria. These studies confirmed the inhibitory effects of the studied plants on bacteria. In this study, the effects of hydroalcoholic *Litsea glutinosa* extract were investigated on *S. mutans* and *Klebsilla p.* An important explanation of medicinal plants inhibiting bacteria is the presence of phenolic compounds in their essential oils and extracts, which enable them play an effective role, thanks to their hydrophobicity, in decomposing cell and mitochondrial membrane lipids and changing membrane permeability and hence bacterial cell death through bonding with amino groups and hydroxylamine proteins.

The difference in the effects due to *Litsea glutinosa* extract between *S. mutans* and *Klebsilla p.* can be related to difference in structures of these two bacteria, the type of culture medium used, and extraction method, and the compounds identified in *Litsea glutinosa*. Moreover, this work confirmed that the antibacterial activity of *Litsea glutinosa* was associated with phenolic, flavonolic, and flavonoid compounds.

Conclusion

Overall, hydroalcoholic *Litsea glutinosa* extract has a high antioxidant property and large amounts of phenolic, flavonolic, and flavonoid compounds, and can inhibit DPPH free radicals. Because antioxidant compounds of plants can exert protective effects on the body's cells, *Litsea glutinosa* extract can be considered an effective factor for human beings' health and used as a nature-based antioxidant to prevent and treat diseases. Besides that, *Litsea glutinosa* extract can be replaced partly with synthetic antioxidants to reduce the risk of liver

damage and development of cancer using nature-based antioxidants. Besides that, this study demonstrated that hydroalcoholic *Litsea glutinosa* extract had antibacterial effects on *S. mutans* and *Klebsilla p*. strains. This finding requires further investigations so that other effective concentration of *Litsea glutinosa* extract can be studied on these two bacteria and clinical strains. Regarding the findings of this work, we can argue that hydroalcoholic extract of *Litsea glutinosa* is a rich source of phenolic, flavonolic, flavonoid, and antioxidant compounds which can be used in food, pharmaceutical and cosmetic industries, as an inhibitory agent of bacterial growth, and a suitable alternative to antibiotics and synthetic drugs.

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