Antioxidant activity, Phytochemical Screening and Antimicrobial properties of *Syzygium lateum* leaves extract.

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Abstract

To investigate the phytochemical composition, in vitro antimicrobial and antioxidant properties of methanolic extracts of Syzygium lateum leaves. Different solvent extracts (Methanol, Choloroform and Ethyl acetate) were prepared from fresh dried leaves of Syzygium lateum by rotator shaker method. The phytochemical screening was carried out number tested in different solvent extract. Methanolic extract of Syzygium lateum were tested for antimicrobial efficacy against Gram positive, Gram negative and fungal organisms. The antimicrobial effect produced by methanolic and chloroform extract of Syzygium lateum was comparable to that of gentamycin was used as standard. The antioxidant activity of methanolic extracts of Syzygium lateum estimated by free radical scavenging activity was determined treating with different concentrations of Vitamin C as standard antioxidant compound. Syzygium lateum was subjected for phytochemical analysis in different solvent extracts which revealed the presence of reducing compounds, alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, phenols and absence of quinone, catachine and coumarine. Methanolic and chloroform extract of Syzygium lateum exhibits antimicrobial activity same as that compared to standard drugs gentamycin. The extract possesses a significant antioxidant potential compared to that of the standards L-ascorbic acid. These results concluded that Syzygium lateum leaves possess high antimicrobial and antioxidant activity and can be used for the development of a safe herbal antioxidant and antimicrobial agents.

Keywords: Syzygium lateum, Phytochemical, Antioxidant and Antimicrobial

I. INTRODUCTION

Human beings are depending on botanicals directly or indirectly for the treatment of various ailments since ancient time, according to the World Health Organization, reports nearly 80% of the world population still depends on traditional medicines to maintain their psychological and physical health [1]. Plant produce a wide variety of secondary metabolites such as vitamins, terpenoides, tannins, flavonoids, alkaloids and other metabolites, which are rich in antimicrobial and antioxidant activity [2]. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno pharmacological information is generally considered as an effective approach in the discovery of new antioxidant and ant-infective agents from higher plants [3]**.** The most active components found in artichoke species consists of flavones, their glycosides, coumarins, sterols caffeoylquinic acids and triterpenoid saponins [4, 5, 6, 7, 8] which are associated with their relative activity against microorganisms.

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Syzygium lateum belong to the family Myrtaceae. It is endemic to southern Western Ghats, central and south Maharastra Sahyadris. Flowers are large, showy, with numerous red stamens flaring out; grow upto 7m tall. Leaves are simple, opposite, carried on 0.5-0.7 cm long stalks. Berry is ovoid, 2.5-3 cm long, crowned with persistent sepals.

The aim of this study was to investigate the *S. lateum* leaves for its phytochemical composition, DPPH radical scavenging activity, Total antioxidant activity and antimicrobial activity.

II. MATERIALS AND METHODS

2.1. Plant material

S. lateum plant was collected from the natural population growing in the Courtallam forest Area, Tirunelveil District, Tamil nadu, India, during October 2015. The plant sample was carried to the Botany Research Laboratory; Voucher specimen of the plant was deposited in the Botany research laboratory V.H.N.S.N.College (Autonomous) for further references.

2.2. Preparation of leaves extracts

The dried leaves extracts were prepared by sequential extraction method using three organic solvents in the basis of polarity of solvents (Chloroform, Ethyl acetate and Methanol). 30g of the fresh leaves sample was taken in a conical flask and 200 ml of Chloroform was added. The conical flask was kept on mechanical shaker for 24 hours, after that the extract was filtered through whatman filter paper No: 1 and the pellet was allowed for drying and this pellet was used for the next solvent extraction (Ethyl acetate and Methanol). The dried extract was recovered and stored in Refrigerator for further analysis.

2.3. Phytochemical Screening

The collected plant leaves extracts were subjected to qualitative, quantitative phytochemical analysis, antioxidant and antimicrobial screening for identification of various classes of active chemical constituents were carried out using standard methods.

2.4. Test for Alkaloids (Mayers' Test)

To 1 ml of leaf extract, 6 drops of Mayer's reagent was added leading to the formation of a yellowish creamish precipitate indicating the presence of alkaloids [9, 10].

2.5. Test for Saponins (Foam Test)

Shake 1ml of leaf extract with 5ml of distilled water and heat it in boiling water bath. Frothing indicates the presence of saponins [9, 10].

2.6. Test for Tannins (Braymers Test)

Take 1ml of the leaf extract with 2 ml of water and add 2 drops of 5% ferric chloride solution. Dirty green precipitate indicates the presence of tannins [9, 10].

2.7. Test for Steroids (Salkowski Test)

To 2ml of the extract, 2 ml of chloroform was added and dissolved. Then add concentrated sulphuric acid. Reddish brown ring at the junction shows the presence of steroids. [11].

2.8. Test for Terpenoids

To 2ml of the extract, 2 ml of acetic acid was added. Then add concentrated sulphuric acid. Deep red coloration shows the presence of steroids [11].

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2.9. Test for Coumarins

Take 2 ml of the extract and add 3 ml of 10% sodium hydroxide. Formation of yellow coloration indicates the presence of Coumarins [11].

2.10. Test for Catachin

Take 2 ml of alcoholic extract solution is treated with a few drops of Echrilich reagent and a few drops of a concentrated HCL. The pink color developed indicates the presence of catachin [12].

2.1.1. Test for Phenols

1 ml of the extract in the test tube was treated with 3% ferric chloride. If the solution turns into deep blue colour, then it shows the presence of phenol [13, 14].

2.1.2. Test for Flavonoids

Treat 1ml of extract 1 ml of sulphuric acid. If the test solution shows orange colour formation conforming the presence of flavonoids [13, 14].

2.1.3. Test for Quinone

Treatment of 1 ml of extract with 5 ml of HCL might result in yellow colour precipitated formation conforming the presence of quinine [13, 14].

2.2.1 Estimation of total phenol content

The amount of total phenol was determined with the Folin–Ciocalteu reagent using the method given by Lister and Wilson, (2001) [15]. This method was employed to evaluate the phenol content of the samples. A standard curve was prepared by using gallic acid as a standard. Different concentrations of gallic acid were prepared in 80% of methanol, and their absorbance was recorded at 760 nm. 100 µl of sample was dissolved in 500 µl (1/10 dilution) of the Folin–Ciocalteu reagent and 1000 µl of distilled water. The solutions were mixed and incubated at room temperature for 1 min. After 1 min, 1500 µl of 20% sodium carbonate (Na_2CO_3) solution was added. The final mixture was shaken and then incubated for 2 h in the dark at room temperature. The absorbance of all samples was measured at 760 nm using a UV–Vis spectrophotometer (Model. U.2800, Hitachi) and the results are expressed in mg of gallic acid equivalents (GAE) per mg of dry weight of the plant. The amount of phenol in plant extracts in gallic acid equivalents (GAE) was calculated by the following formula:

$$X = (A.\ mo) / (Ao.m)$$

Where X is the phenol content, mg/mg plant extract in GAE, A is the absorption of plant extract solution, Ao is the absorption of standard gallic acid solution, m is the weight of plant extract, and mo is the weight of gallic acid in the solution.

2.2.2. Free-Radical Scavenging Ability (DPPH-assay)

The scavenging ability of methanol extract on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free-radicals was estimated according to the method of Shimada *et al*., (1992) [16]. This method depends on the reduction of purple DPPH to a yellow coloureddiphenylpicrylhydrazine and the colour was measured at 517 nm. About 2 ml of various concentrations (10- 250µg/ml) of test sample was mixed with 0.5 mL of 0.01 mM DPPH in methanol. An equal amount of methanol and DPPH served as control. The mixture was shaken vigorously and then steadily stayed for 30 min at room temperature in dark. The absorbance of the resulting solution was measured at 517 nm against the blank using a UV–Vis spectrophotometer (Model. U.2800, Hitachi). The experiment was performed in triplicates. The DPPH radical scavenging activity was calculated according to the following equation:

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% DPPH radical scavenging activity = (Ao – A1)/ Ao × 100%

whereAo is the absorbance of the control reaction and A1 is the absorbance in the presence of the sample of the tested extracts. Percentage radical activity was plotted against the corresponding antioxidant substance concentration to obtain the IC_{50} value, which is defined as the amount of antioxidant substance required to scavenge the 50% of free radicals present in the assay system. IC_{50} values are inversely proportional to the antioxidant potential.

2.2.3. Total antioxidant activity by Phosphomolybdenum method

Total antioxidant capacity was expressed as ascorbic acid equivalent and was calculated using the Phosphomolybdenum method (Prieto and Pineda, 1999) [17]. Antioxidant present in the sample reduce the Mo(VI) to Mo(V) which then react with the phosphate group sodium phosphate to form a green coloured Mo(V) – Phosphate complex (Phosphomolybdenum complex) in an acetic medium. This complex is then spectophotometrically measured at 695 nm (Model. U.2800, Hitachi). The tubes containing 0.2 ml of extract (10-200µg/ml) is mixed with 1.8 ml of distilled water, 2ml of Phosphomolybdenum reagent solution. Incubate it at 95ºC for 90 minutes. The mixture is closed to room temperature and the absorbance is measured at 695 nm against reagent blank. The total antioxidant capacity was expressed as equivalents of Ascorbic Acid by using the standard Ascorbic Acid graph.

2.2.4. Antimicrobial activity

Test Organisms:

Staphylococcus aereus, S*treptococcus faecalis, Bacillus subtilis, Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and fungus *Candida albicans* (Micro organisms were collected from the stock of the Dept of Biology GRU, Dindugul.).

The test organisms were maintained on nutrient agar slant and kept in a refrigerator at 4oC. 100ml aliquots of nutrient broth were inoculated with the culture of test micro-organisms using aloop and then incubated at 37°C for 24 hrs.

Antimicrobial activities of methanol, ethyl acetate and chloroform fractions of *S.lateum* were carried out using the agar well diffusion method. The stock was maintained on nutrient agar slant and sub cultured in nutrient broth for incubation at 37°C prior to each antimicrobial testing. Mueller-Hinton agar medium (MHA) was used for antimicrobial susceptibility tests. The MHA medium was prepared by pouring 20 ml of molten media into sterile Petri plates. The plates were allowed to solidify and 0.2ml of an overnight broth culture of test micro-organisms was added to 20ml of cooled molten agar was swabbed uniformly on the medium and allowed to dry for 5 min. For agar well diffusion method, four equidistant wells (6 mm in diameter) were cut from the agar with the help of a cork-borer. 40 µl of leaves extracts (methanol, ethyl acetate and Chloroform extracts) containing (4 mg) concentration was loaded on 6 mm well. The standard antibiotic disc gentamicin (10 µg) was placed on the surface of the plates. The plates were kept for incubation for 24 hrs at 37°C. The zone of inhibition was measured around the well containing samples and standard. The experiments were performed in triplicates.

2.2.5. Statistical Analysis

All analysis was carried out in triplicates. The results of scavenger activity and total phenolic and total flavonoid contents were performed from the averages of all samples reading Mean+- SD used Excel 2003.

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III. Results and Discussion

3.1. Qualitative phytochemical analysis

Phytochemical Screening the solvent extracts of *S. lateum* revealed the presence of secondary metabolites such as phenols, alkaloids, flavonoids, saponins, tannins and steroids (Table 1). The results also revealed that, terpenoid, quinine and catachine were absent in all the solvent extracts of the plant.

Table.1 Qualitative Phytochemical Analysis of *S. lateum* leaf extracts

Plant solvent extraction	Alkoloid	Flavonoid	Phenol	Saponin	Tannin	Terpenoid	Quinone	Catechin	Coumarin	Steroid
Chloroform	-	+	-	+	-	-	-	-	-	+
Ethyl acetate	+	+	+	-	+	-	-	-	-	+
Methanol	-	+	+	-	+	-	-	-	+	-

+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals.

3.2. Quantitative Phytochemical Analysis

The quantitative phytochemical content determination of the three solvent fraction extract of *S. lateum* result as given in Table 2. indicates that the phenol content in the methanol extracts of *S. lateum* (0.559±0.04mg/mg), Chloroform extract (0.366±0.04mg/mg) and Ethyl Acetate Extract (0.388±0.02mg/mg). *S. lateum* extracts had the most significant amount of phenol conent in methanol extracts of 0.559 mg/mg when compared to other solvent extracts.

Table. 2. Quantitative phytochemical analysis of three solvent extracts of *S. lateum*

Phytochemical constituent	Mehanol extract (mg/mg)	Ethyl acetate extract (mg/mg)	Chloroform extract (mg/mg)
Phenol	0.559±0.04	0.388±0.02	0.366±0.04

All values are mean ± standard deviation (n = 3).

3.1.1. DPPH radical scavenging activity

Figure.1 shows the results of the free radical (DPPH) scavenging activity in % inhibition. The result shows that the methanol fraction of *S. lateum* exhibited the highest radical scavenging activity with 79.99±.2.31. The results revealed that free radical scavenging activity of methanolic leaf extracts of *S. lateum* has significant radical scavenging ability on DPPH with IC_{50} value of 44.48µg/ml respectively. The positive control ascorbic acid showed the IC_{50} values of 29.68 µg/ml.

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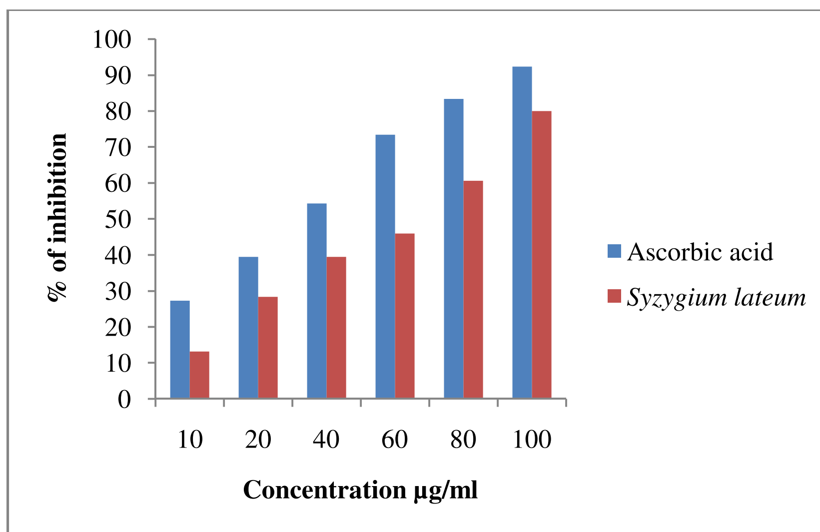

Fig.1 Antioxidant activity by DPPH method of methanolic extract of *S. lateum* at different concentrations. Values are mean ± S.D. of three replicates.

3.1.2. Total phospomolybdenum method

The phosphomolybdate method is quantitative, since the total antioxidant capacity (TAC) is expressed as ascorbic acid equivalents. The results showed antioxidant activity in dose dependent manner at concentration 25 to 250 µg/ml. The methanol extract of *S. lateum* exhibited significant antioxidant activity of 1.639 mg AA equivalent /mg of dry weight of sample. Strong antioxidant activity of methanol statistically similar to ascorbic acid indicates strong antioxidants in this fraction and these could be attributable to the presence of phenolic compounds.

3.1.3. Antimicrobial activity

Table. 3. shows Antimicrobial activity *of* S. lateum methanol, ethyl acetate and chloroform extracts was screened against *B. subtilis, S. aureus, S. faecalis, K. pneumoniae*, *E. coli* and *P. aeruginosa, C. albicans*. The results of the zone of inhibition are summarized in Table 3. The maximum zone of inhibition noted in methanol extract against *Escherichia coli* (35mm) *Streptococcus faecalis* (31mm), *Klebsiella pneumonia* and *Bacillus subtilis* (30mm). Chloroform and Ethyl acetate extract shows highly active against all the pathogens in the table. All the values are compared with gentamicin positive control.

Table.3 Antimicrobial activity of *S. lateum* leaf extracts against test microbes.

Microbial strain	Solvent Extracts (Zone of inhibition in mm)			
	Methanol	Ethyl acetate	Chloroform	Gentamicin
Bacillus subtilis	30	19	26	16
Klebsiella pneumoniae	30	15	24	25
Candida albicans	25	17	23	20
Pseudomonas aeruginosa	25	16	25	33
Staphylococcus aureus	25	16	25	18
Escherichia coli	35	17	29	26
Streptococcus faecalis	31	16	24	27

*Each value represents Mean ± SD of three replicates

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Phytochemical screening is an essential and very important part of medicinal plants research. Alkaloids, tannins, saponins, steroid, terpenoids, flavonoids, phenolic compounds and cardio glycoside distribution in five medicinal plants belonging to different families were assessed and compared [18]. In the present studies, the results of various phytochemical analytical methods have convincingly proved that *S. lateum* extracts were found to have good amounts of phenols, alkaloids, flavonoids, saponins, tannins and steroids.

Several studied done all over the world have proved that phenolic compounds are having exceptional antioxidant potential which makes many plants as medicinal plants [19]. The conception of antioxidant action of phenolic compounds is not novel [20]. Various antioxidant and free radical scavenging activities carried out in the present study for the extracts of *S. lateum* clearly indicated that the plant contains valuable medicinal properties to be used as potent antioxidant compounds. Phytochemical screening of *S. lateum* extracts revealed very clearly that they contain good amounts of phenolics. Thus it has been concluded that the adequate presence of phenolics in *S. lateum* makes them a potent antioxidant medicinal plant. Further analysis on the mechanism of these antioxidants and other beneficial compounds from *S. lateum* are needed for a better understanding to implement them as potent candidates for antioxidant drugs.

The presences of these secondary metabolites are known to have therapeutic activity against several diseases and therefore could suggest its traditional use for the treatment of various illnesses [21]. Earlier studies have reported that flavonoids have antibacterial property as they have the capability to associate with soluble proteins and bacterial cell walls [22]. These flavonoids also have antioxidant property as they inhibit oxidative and hydrolytic enzymes, have impact on radical scavenging, anti-inflammatory and anti-cancerous activity [23, 24]. Similarly saponins are reported to act adversely on bacteria and fungi so they are reported to have antimicrobial [25], while alkaloids are used for their antiparasitic, antioxidant, anticancerous and antimicrobial activity [24] and tannins are reported to have antimicrobial, antidiarrhhoel, anti-inflammatory, antioxidant activities and have astringent property [26].

IV. CONCLUSION

The phytochemical tests indicated the presence of phenols, alkaloids, flavonoids, saponins, tannins and steroids in the plant extract. Several of such compounds are known to possess potent antioxidant activity. The results of antioxidant activity indicate higher free radical scavenging activity in methanolic extracts of *S. lateum* due to presence of phytochemical constituents specially phenolic compounds. Further research is needed towards isolation and identification of active principles present in the extracts which could be used for pharmaceutical use.

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