Available online at www.scholarsresearchlibrary.com

Scholars Research Library

J. Nat. Prod. Plant Resour., 2016, 6 (4):15-19
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Antimicrobial and Phytochemical analysis of different solvent extracts of barks of *Syzygium laetum* (Buch.-Ham.) Gandhi

Kokkaiah Irulandi, Sethupandian Geetha, Murugesan Suresh, Vadivel Siva, Natarajan Nirmalkumar and Palanichamy Mehalingam*

Research Department of Botany, V.H.N. Senthikumara Nadar College (Autonomous), Virudhunagar

---

ABSTRACT

The present study was carried out to evaluate the phytochemical and antibacterial activity of methanol, ethyl acetate and acetone extracts of Syzygium laetum bark. Antibacterial activity was analysed by agar well diffusion method against gram positive and gram negative bacteria. The phytochemical analysis carried out revealed the presence of alkaloids, coumarins, flavanoids, phenols, saponins, terpenoids, tannins and steroids in various solvent extracts. Methanolic extract of S. laetum showed good antibacterial activity against Bacillus subtilis, Pseudomonas aeruginosa and Klebsiella pneumoniaee. Acetone extract revealed similar zone of inhibition activity against Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus faecalis, Klebsiella pneumoniae and Bacillus subtilis where as ethyl acetate extract showed least zone of inhibition against tested all pathogens compared with other solvent extracts. From our results showed the presence of crucial phytoconstitients and good antibacterial activity and suggest that S. laetum can be used in the microbial tests.

Keywords: *Syzygium laetum*, phytochemical analysis, Antibacterial activity, Agar well diffusion method.

INTRODUCTION

Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for discovering new drugs. Extracts of many plants are highly efficient against parasitic as well as microbial infections. It is estimated that around 70,000 plant species from lichens to tall trees, have been used at one time to other for medicinal purposes [1] .The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes [2]. Numerous studies have identified compounds within herbal plants that are effective antibiotics [3]. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics [4].

Syzygium laetum (Buch. - Ham.) Gandhi is a medium sized tree grows up to 7m belongs to the family Myrtaceae. It is widely dispersed in the Western Ghats and Evergreen Forests. Endemic to Southern Western Ghats. The plants having white color bark smooth; blaze brown. Leaves simple, opposite, decussate, Inflorescence in terminal or axillary divaricate cymes or solitary. Fruits are Berry, ovoid, 2.5 - 3 cm long, crowned with persistent calyx. December-July is the flowering and fruiting seasons. A perusal of literature revealed that no work has been carried out on the phytochemical analysis and antimicrobial activity of barks extracts of *S. laetum*. In the present study, an

attempt was made to screen different multi solvent extracts prepared from dried bark of *Syzygium laetum* to study the antimicrobial activity and phytochemical analysis.

MATERIALS AND METHODS

Plant material

The bark of *Syzygium laetum* were collected from the natural population growing in the Courtallam forest Area, Tirunelveli district, Tamil Nadu, India, during July 2015. The plant sample was carried to the Botany Research Laboratory and voucher specimen of the plant was deposited in the Botany research laboratory of V.H.N.S.N.College (Autonomous) for further references.

Preparation of bark extracts

The plant bark were cleaned, washed, shade dried and powdered for the phytochemical studies and extraction method using an organic polar solvent methanol. 40g of the dried bark sample was taken in a conical flask and 300ml of methanol was added. The conical flask was kept on mechanical shaker for 24 hours, after which the extract was filtered through Whatman No: 1 filter paper in to a glass beaker and then allowed to dry by incubation in an oven at 64.7ºC. The dried extract was recovered and stored in refrigerator for further analysis.

Phytochemical Screening

Qualitative phytochemical analysis

The dried bark extract was subjected to Antimicrobial activity and qualitative phytochemical screening for the identification of various classes of active biochemical constituents.

Test for Alkaloids (Mayers' Test)

To 1 ml of bark extract, 6 drops of Mayer's reagent was added. The formation of yellowish creamish precipitate indicated the presence of alkaloids [5, 6].

Test for Saponins (Foam Test)

1ml of bark extract was mixed with 5ml of distilled water. The contents were heated in a boiling water bath. Frothing indicated the presence of saponins [5, 6].

Test for Tannins (Braymers Test)

1ml of the bark extract was mixed with 2 ml of water. To this, 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins [5, 6].

Test for Steroids (Salkowski Test)

To 2ml of the bark extract, 2 ml of chloroform was added followed by concentrated sulphuric acid. Formation of reddish brown ring at the junction showed the presence of steroids. [7].

Test for Terpenoids

2ml of the bark extract was treated with 2 ml of acetic acid. Then concentrated sulphuric acid was added. The formation of deep red color showed the presence of steroids [7].

Test for Coumarins

2 ml of the bark extract was taken and 3 ml of 10% sodium hydroxide was added. The development of yellow coloration indicated the presence of Coumarins [7].

Test for Catechins

2 ml of bark extract solution was treated with few drops of Echrilich reagent and few drops of concentrated HCL. The appearance of pink color indicated the presence of catechins [7].

Test for Flavonoids

1ml of the bark extract was added with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids [8, 9].

Test for Quinones
1ml of the bark extract was treated with 5ml of HCL. Formation of yellow color precipitate indicated the presence of quinones [8, 9].

Test for Phenols
1 ml of the bark extract was treated with 3% ferric chloride. The appearance of deep blue color, showed the presence of phenols [8, 9].

ANTIBACTERIAL ACTIVITY
Staphylococcus aureus, Streptococcus faecalis, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae. All samples were kindly donated by the Dept of Biology GRU, Dindigul.

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

Antibacterial activities of different solvent extracts of plants 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 100µl 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 bark extracts (methanol, acetone and ethyl acetate extracts) containing (4 mg) concentration was loaded on 6 mm well. The standard antibiotic solution 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.

Statistical analysis
All the data was reported as Mean ± Standard Deviation of three replicates. Statistical analysis was performed using Microsoft Excel.

RESULTS

The result of preliminary phytochemical screening of different extract of barks of *S. laetum* is presented in table 1. The results revealed the presence of phytoconstituents like alkaloids, coumarins, flavanoids, phenols, saponins, terpenoids, tannins and steroids in the crude extracts of *S. laetum* bark. The phytoconstituents catechins and quinones were not reported in all the tested solvent extracts. A total of eight phytoconstituents were reported from the methanolic extract. It includes alkaloids, flavonoids, coumarins, phenols, steroids, tannins, saponins, terpenoids. The phytoconstituent steroids were reported in all the extracts, but the alkaloids were reported only in methanol and ethyl acetate extracts. Tannin also negative test for the acetone and ethyl acetate extracts.

Table . 1. Preliminary phytochemical screening of different extract of *S. laetum* bark

Plant extracts	Alkaloids	Catechins	Coumarins	Flavonoids	Phenols	Quinones	Saponins	Steroids	Tannins	Terpenoids
Acetone	-	-	-	+	-	-	-	+	-	+
Ethyl acetate	+	-	-	-	+	-	-	+	-	-
Methanol	+	-	+	+	+	-	+	+	+	+

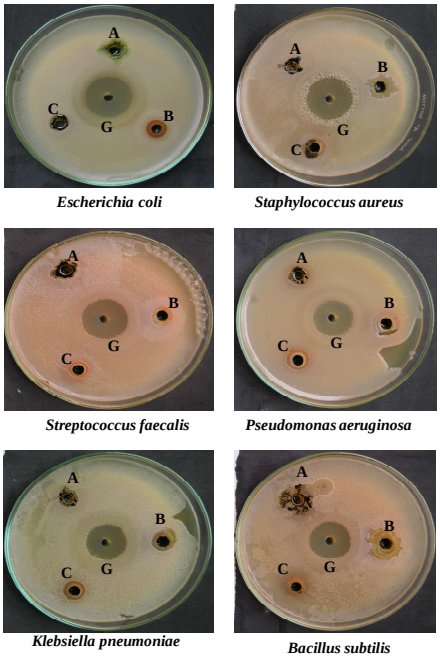
+ = indicates presence of phytochemicals, - = indicates absence of phytochemicals.

Results showed in the present study relieved that the tested bark extracts of *S. laetum* posses potential antibacterial activity against *Streptococcus feacalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis, Staphyllococcus aureus,* and. (Table 2 and Fig. 1). When tested by the well diffusion method, the methanol leaf extract of *S. laetum* showed significant activity against *B subtilis* (13.5±0.57mm), *P. aeruginosa* (12.5±0.50mm) and *K. pneumoniae* (11.2±0.9mm). The acetone extract showed maximum antibacterial activity against the all the tested microbes ranges between (10 to 11.66mm). The ethyl acetate extract of *S. laetum* showed not activity against the *E. coli*. The ethyl acetate extract revealed minimum percentage of activity against tested cultures.

Table 2. Antimicrobial activity of three solvent extracts from the bark of *S. laetum*

Microbial strain	Solvent Extracts (Zone of inhibition in mm)			
	Methanol	Acetone	Ethyl acetate	Gentamicin
Escherichia coli	8.5±0.50	10±0.00	0.0±0.00	21±1.00
Staphylococcus aureus	10±1.00	11.5±0.57	10.33±0.57	22±1.00
Streptococcus faecalis	9±0.00	10.5±0.57	8.33±0.50	21.9±0.23
Pseudomonas aeruginosa	12.5±0.50	12±0.00	10.66±1.15	21.33±0.57
Klebsiella pneumoniae	11.2±0.9	11.66±1.15	8.33±0.57	19.33±0.57
Bacillus subtilis	13.5 ±0.57	11±1.00	8.33±0.57	23.33±0.57

**Each value represents Mean ± Standard deviation of three replicates*



A= Methanol, B= Acetone, C= Ethyl acetate, G=Gentamicin

Fig 1. Antibacterial activity of three solvent extracts of *Syzygium lateum* bark

DISCUSSION

Phytochemical constituents such as alkaloids, glycosides, reducing sugar, flavonoids, tannins, saponins, and several other organic compounds are secondary metabolites of medicinal plants that serve as defense mechanism against many microorganisms and insects [10]. The investigated plants did not show strong antibacterial activity; however, negative results do not mean absence of bioactive constituents nor is that the plant inactive. Active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed [11]. Lack of activity can thus only be proven by using large doses [12]. The leaves of Syzygium cumuni showed significant amount of antibacterial activity. The methanolic bark extract revealed good antibacterial activity the aqueous extract and considerable amount of phytoconstituents presents in the methanol extract. These results revealed the presence of phytocompounds have extract with in methanol, which is similar to our results.

CONCLUSION

The acetone extracts of barks of *Syzygium laetum* exhibited the most remarkable antimicrobial activity than other solvent extracts. However, methanolic bark extracts showed predominant amount of phytoconstituents than the other

solvent extracts. This plant can be further subjected to pharmacological investigation for the development of new potential antimicrobial compounds.

Acknowledgement
The corresponding author is grateful to University Grants Commission, New Delhi for providing financial assistance under Research Award Scheme.

REFERENCES

[1] SS Purohit, SP Vyas, 2004, 1st edition. *Publishers Agrobios*, Jodhpur, India, **2004**, 1-3.
[2]AJ Afolayan, *Pharm. Biol*, **2003**, 41: 22-25.
[3]A Basile, S Sorbo, S Giordano, L Ricciardi, S Ferrara, D Montesano, R Castaldo Cobianchi, ML Vuotto, L Ferrara, *Fitoterapia*, **2003**, 71: 110-116.
[4]T Okpekon, S Yolou, C Gleye, F Roblot, P Loiseau, C Bories, F Grellier, F Frappier, A Laurens, R Hocquemiller, *J. Ethnopharmacol*, **2004**, 90: 91-97.
[5]HO Edeoga, DE Okwu, BO, Mbaebie, *Afr J Biotechnol*, **2005**, 4: 685-688.
[6]JB Harbone, *Chapman and Hall Ltd*, London, **1973**, 188.
[7]M Yaday, S Chatterji, SK. Gupta, G Watal, *IJCPR*, **2012**, 4(3): 0975-7066.
[8]KC Kokate, Vallabh Prakashan, Delhi, **2000**, 107-111.
[9]B Harbone, *Chapman and Hall*, London, **1999**, 60-66.
[10]G.H.S Bonjar, AK Nik, S Aghighi, *J Biol Sci*, **2004**, 4: 405-412.
[11]JLS Taylor, T Rabe, LJ McGraw, AK Jager, J van Staden, *Plant Growth Regulator*, **2001**, 34: 23-37.
[12]NR Farnsworth, In: Rasoanaivo P, Ratsimamanga-Urverg S (Eds) Biological Evaluation of Plants with Reference to the Malagasy, Flora, Madagascar, **1993**, 35-43.