ORIGINAL ARTICLE

Antimicrobial, antioxidant, anticancer activities of *Syzygium caryophyllatum* (L.) Alston

Gayathri Annadurai, Benish Rose Pious Masilla, Saranya Jothiramshekar, Eganathan Palanisami, Sujanapal Puthiyapurayil[1], Ajay Kumar Parida[2]

Plant Tissue Culture and Bioprospecting Laboratory, [2]Plant Molecular Biology, M.S. Swaminathan Research Foundation, 3rd Cross Road, Taramani, Chennai, [1]Community Agro Biodiversity Centre, M. S. Swaminathan Research Foundation, Wayanad, Kerala, India

Background: *Syzygium caryophyllatum* (L.) Alston is an endangered tree species belonging to the Myrtaceae family. **Objective:** To evaluate the antimicrobial, antioxidant and anticancer activities of the leaf extract. **Materials and Methods:** Disc diffusion method was used for antimicrobial screening of four bacterial and three fungal strains. Scavenging ability of the extract was determined using 2,2-diphenyl-1-picrylhydrazyl assay. Hep2 cell line was used to evaluate the cytotoxicity by 3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide assay. **Results:** The zone of inhibition was high for the extract against all the selected strains used. Antioxidant potential was more at higher concentration (400 µg/ml). The extract showed maximum Hep2cell inhibition at higher concentration (9.80% cell viability in 1000 µg/ml). **Conclusions:** Antioxidant, antimicrobial and anticancer studies using leaves of *Syzygium caryophyllatum* showed that it has potential utilization in pharmaceutical industry.

Key words: Anticancer, antimicrobial, antioxidant, myrtaceae, *Syzygium caryophyllatum*

INTRODUCTION

Natural products and their derivatives have historically been exploited as a valuable source of novel therapeutic agents.[1] Further, a large proportion of plant based compounds are used as lead molecules in drug discovery to produce synthetic molecular analogs, implying that phytochemicals play a critical role in diversity-oriented synthesis (DOS) of natural product-like pharma-compounds.[2]

Syzygium caryophyllatum is one of the species that has been categorized as endangered tree species under the international nature for conservation of nature (IUCN)[3] red list of threatened species. *Syzygium* is a genus of flowering plants comprising of about 1200 species, having a native range in tropical Africa, subtropical to tropical Asia, Australia, New Caledonia, New Zealand, Pacific islands.[4] 80 species are reported from China[5] and more than 75 species from India.[6] *Syzygium* species exhibit antidiabetic,[7] antifungal,[8-10] anti-inflammatory,[11] antibacterial,[10,12] antioxidant,[10,13] antihyperlipidemic,[14] and growth inhibitory effects against oral pathogens.[15] The *Syzygium* species are also found to posses antihyperglycemic activity,[16] cytotoxic,[10,17] anti-angiogenic,[17] and anti-nociceptive activity.[18] To the best of our knowledge no study on the biological activity of *Syzygium caryophyllatum* is reported so far. Thus, in the present study, the antimicrobial, antioxidant and cytotoxicity of the extract were evaluated.

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.108210

MATERIALS AND METHODS

Plant material of *Syzygium caryophyllatum* was collected from Pozhuthana, Wayanad district in the state of Kerala in India, during September 2010, and identified as *Syzygium caryophyllatum*. Freshly harvested 1 kg leaves of *S. caryophyllatum* were shade dried at room temperature until disappearance of moisture. Dried leaves were grounded to fine powder using a table top mixer. 400 g of ground powder was added with 900 ml of ethyl acetate and kept at room temperature for 12 hours with 6-8 shakes. Compound extraction was carried out using Soxhlet apparatus[19] at 77°C for 4 hours. The solvent was collected and evaporated at 77°C using a Rotary Evaporator. Colloidal stage of extract with ethyl acetate was collected from Soxhlet and dried under laminar air flow and dried extract kept under 4°C until use.

Four bacterial strains (*Aeromonas hydrophila*, *Staphylococcus aureus*, *Bacillus subtilis* and *Entercoccus faecalis*) and three fungal (*Aspergillus niger*, *Alternaria*

Address for correspondence: Dr. Eganathan Palanisami, Plant Tissue Culture and Bio-prospecting Laboratory, M.S. Swaminathan Research Foundation, 3rd Cross Road, Taramani, Chennai - 600 113, India. E-mail: eganathan@gmail.com
Received: 05-06-2012; **Accepted:** 21-12-2012

alternata and *Pencillium chrysogenum*) species were used in the study. The bacterial stock culture were maintained on nutrient agar medium and fungal culture on potato dextrose agar medium, stored at 4°C.

The extracts were tested for their antimicrobial activity using disc diffusion method. Bacterial species were sub-cultured on nutrient agar medium and fungal species on potato dextrose agar medium, which were then incubated at 37°C for 24 hours and 27°C for 48 hours respectively. Test solutions of dried extracts at concentrations of 1000 µg, 500 µg, 250 µg, 100 µg/ml were impregnated on sterile discs. Ampicillin and Nystatin were used as positive controls. The disc impregnated with ethyl acetate was used as negative control. The discs were placed on the surface of the nutrient agar for bacteria and incubated at 37°C for 24 hours, and on the surface of the potato dextrose agar for fungi and incubated at 27°C for 48 hours. Inhibition zones were calculated as the difference between disc diameter (6 mm) and the diameters of inhibition.[20] Antibacterial activities were evaluated by determining minimum inhibitory concentration using micro broth dilution assay.[21]

The free radical scavenging activity was evaluated by measuring the scavenging activity of the sample on 2,2-diphenyl-1-picrylhydrazyl (DPPH).[22] Using methanol as a solvent, sample was prepared to the concentration of 1 mg/ml. Pure methanol was used as blank and butylated hydroxyl toluene (BHT) was taken as the standard. 2.7 ml of methanol, 100 µl of sample and 200 µl of DPPH reagent (1 mg/ml) were added and these mixtures kept in dark incubation at RT for 30 mins. Samples were visualized in UV-VIS spectrophotometer at a wavelength of 517 nm.

Percentage of DPPH radical scavenging activity of the sample was calculated as:

$$= [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$$

Where,
A_S is the absorbance of the solution with sample extract is added at a particular level
A_{DPPH} is the absorbance of the DPPH solution.

Human liver carcinoma cell lines (HEp2) purchased from National Centre for Cell Science, Pune was used for the study. The cell line was grown and maintained in a humidified incubator at 37°C and in 5% CO_2 atmosphere. Minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), 100 units/ml penicillin and 100 lg/ml streptomycin was used for the cell culture of HEp2.

Anticancer activity of the sample was measured using 3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay.[23] The monolayer culture of Hep2 cells at a concentration of 10 cells/ml/well were seeded in 24 well titre plates. The cells were permitted to adhere for 24 hours, and the growth medium (MEM) removed using micropipette and the monolayer of cells washed twice with MEM without FBS to remove dead cells and excess FBS. 1ml of medium (without FBS) containing different dilution of drugs were added in respective wells; 200 µl of MTT (5 mg/ml in PBS) were added to each well, and the cells incubated for a further 6-7 hrs in 5% CO_2 incubator. After removal of the medium, 1ml of DMSO was added to each well. Absorbance was recorded at the wavelength of 570 nm. The effect of extracts on cell growth inhibition was assessed as percent cell viability, where vehicle-treated cells were taken as 100% viable.

Percentage of viable cell concentration was calculated thus:
Viability (%) = [(Mean OD/Control OD)-1] ×100

Mean value of antimicrobial and anticancer were calculated for three assays. Antioxidant assay was repeated three times and calculated mean ± standard error.

RESULTS AND DISCUSSION

Antimicrobial activity of ethyl acetate extract of *S. caryophyllatum* is presented in Table 1. The extract of *S. caryophyllatum* was tested with different concentrations (100 to 1000 µg/disk) that have produced a maximum zone against *Staphylococcus aureus* and minimum zone against *Enterobacter faecalis* and antifungal activity effective against *Alternaria alternata*. The zone of inhibition gradually increased on increasing the concentration of the extract for all the strains used. MICs were performed with the same extracts and the results tabulated in Table 2.

The ethyl acetate extract of *S. caryophyllatum* leaves

Table 1: The antimicrobial activities of *Syzygium caryophyllatum* leaf extract

Test microorganisms	Zone of inhibition (mm)*						
	1000 µg	500 µg	250 µg	100 µg	EA µg	St 10 µg	Nystatin
Staphylococcus aureus	11.0	10.0	9.5	9.0	-	21.0	-
Aeromonas hydrophila	10.0	10.0	9.0	8.0	-	21.0	-
Bacillus subtilis	10.0	9.5	9.0	6.0	-	26.0	-
Entercoccus faecalis	9.5	9.0	8.5	-	-	19.0	-
Penicillium chrysogenum	9.5	8	6.6	5.2	-	-	24.0
Alternaria alternate	11.0	9.2	5.5	-	-	-	22.0
Aspergillus niger	10.0	9.4	6.2	-	-	-	21.0

EA – Ethyl acetate; St – Streptomycin; - – no zone of inhibition, *Values are the means of three assays

Table 2: Minimum inhibitory concentrations of *Syzygium caryophyllatum* leaf extract

Test microorganisms	Minimum inhibitory concentration (µg/ml)
S. aureus	500
A. hydrophila	250
B. subtilis	250
E. faecalis	100
Penicillium chrysogenum	100
Alternaria alternate	100
Aspergillus niger	100

Values are the means of three assays

Table 3: Results of the free radical scavenging activity of *Syzygium caryophyllatum* leaves

Sample	Concentration (µg/ml)	%Antioxidant activity
Blank	0	0
Standard	400	97.35±0.13
Sample	50	28.76±0.15
	100	35.68±0.12
	200	56.92±0.11
	400	86.14±0.14

Values are mean and standard error of three replicates

Table 4: Results of the cytotoxicity against Hep2cell lines of *Syzygium caryophyllatum* leaves

Concentration (µg/ml)	% Cell viability
1000	9.80
500	27.45
250	47.05
125	52.94

Values are the means of three assays

were found to act as potent free radical scavengers in comparison with BHT, a commercial antioxidant. At higher concentration (400 µg/ml) the extract has significant inhibition of DPPH radical scavenging activity [Table 3]. This extract has comparatively high activity compared to that of *S. cumini*[24] and are equivalent to that of *Syzygium aromaticum*.[13]

MTT assay was used to evaluate the effect of the extract on cell viability of Hep2cell lines. The ethyl acetate extract treatment to these cell lines resulted in a remarkable dose-dependent inhibition of cell growth. The extract showed maximum cell inhibition at higher concentration [Table 4]. The extract of *Syzygium caryophyllatum* showed higher activity on Hep2 cell lines compared to the activity exhibited by *Syzygium cumini* on AML cells.[24]

Thus from the present study it was proven that the leaves of *Syzygium caryophyllatum* posses effective biological properties against pathogens like *Staphylococcus aureus* that cure skin infections and cancer cells. In addition also it exhibits high scavenging activity against free radical comparable with that of standard available drugs.

REFERENCES

1. Koehn FE, Carter GT. The evolving role of natural products in drug discovery. Nat Rev Drug Discov 2005;4:206-20.
2. Marcaurelle LA, Johannes CW. Application of natural product-inspired diversity-oriented synthesis to drug discovery. Prog Drug Res 2008;66:187-216.
3. IUCN. *Syzygium benthamianum*. In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.1. Available from: http//:www.iucnredlist.org. [Last accessed on 2011].
4. Elliot R, Jones D. Encyclopaedia of Australian Plants Suitable for Cultivation. Port Melbourne: Lothian Press; 2010.
5. Jie C, Craven LA. *Syzygium*. Flora China 2007;3:335.
6. Anand A, Srinivasa Rao C, Balakrishna P. *In vitro* propagation of *Syzygium travancoricum* Gamble-an endangered tree species. Plant Cell Tiss Org Cul 1999;56:59-63.
7. Kumar A, Ilavarasan R, Jayachandran T, Deecaraman M, Aravindan P, Padmanabhan N, *et al*. Anti-diabetic activity of *Syzygium cumini* and its isolated compound against streptozotocin-induced diabetic rats. J Med Plants Res 2008; 2:246-9.
8. Park MJ, Gwak KS, Yang I, Choi WS, Jo HJ, Chang JW. Antifungal activities of the essential oils of *Syzygium aromaticum* (L.) Mer. Et Perry and *Leptospermum petersonii* Bailey and their constituents against various dermatophytes. J Microbiol 2007; 45:460-5.
9. Ayoola GA, Lawore FM, Adelowotan T, Aibinu IE, Adenipekun E, Coker HA, *et al*. Chemical analysis and antimicrobial activity of the essential oil of *Syzygium aromaticum* (Clove). Afri J Microbiol Res 2008;2:162-6.
10. Kiruthiga K, Saranya J, Eganathan P, Sujanapal P, Parida A. Chemical composition, antimicrobial, antioxidant and anticancer activity of leaves of *Syzygium benthamianum* (Wight ex Duthie) Gamble. J Biol Active Prod Nat 2011;1:273-8.
11. Chaudhuri AK, Pal S, Gomes A, Bhattacharya S. Anti-inflammatory and related actions of *Syzygium cuminii* seed extract. Phytother Res 1990;4:5-10.
12. Shyamala GS, Vasantha K. Phytochemical screening and antibacterial activity of *Syzygium cumini* (L.) (Myrtaceae) leaves extracts. Int J PharmTech Res 2010;2:1569-73.
13. Nassar MI, Gaara AH, Ghorab AH, Farrag AR, Shen H, Huq E, *et al*. Chemical constituents of clove (*Syzygium aromaticum*, Fam. Myrtaceae) and their antioxidant activity. Rev Latinoameri de Quim 2007;35:47-57.
14. Modi Dikshit C, Rachh PR, Nayak BS, Shah BN, Modi KP, Patel NM, *et al*. Antihyperlipidemic acitivity of *Syzygium cumini* Linn. Seed extract on high cholesterol fed diet rats. Int J Pharm Sci 1990; 1:330-2.
15. Cai L, Wu CD. Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. J Nat Prod 1996;59:987-90.
16. Rekha N, Balaji R, Deecaraman M. Antihyperglycemic and antihyperlipidemic effects of extracts of the pulp of *Syzygium cumini* and bark of *Cinnamon zeylanicum* in streptozotocin-induced diabetic rats. J Appl Biosci 2010;28:1718-30.
17. Aisha AF, Nassar ZD, Siddiqui MJ, Abu Salah KM, Alrokayan SA, Ismail Z, *et al*. Evaluation of anti-angiogenic, cytotoxic and antioxidant effects of *Syzygium aromaticum* L. extracts. Asian J Biol Sci 2011;4:282-90.
18. Avila Pena D, Pena N, Quintero L, Suarez Roca H. Antinociceptive

activity of *Syzygium jambos* leaves extract on rats. J Ethnopharmacol 2007;112:380-5.
19. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extract of *Acalypha wilkensiana*. J Ethnopharmacol 1993;39:171-4.
20. Hewitt W, Vincent S. Theory and application of Microbiological assay. San Diego: Academic Press; 1989.
21. CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: Approved Standard- 8th ed. CLSI document M07-A8, Wayne, Pennsylvania: Clinical and Laboratory Standards Institute; 2009.
22. Barros L, Baptista P, Ferreira IC. Effect of *Lactarius piperatus* fruiting body maturity stage on antioxidant activity measured by several biochemical assays. Food Chem Toxicol 2007;45:1731-7.
23. Mosmann T. Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. J Immunol Methods 1983;65:55-63.
24. Afify AM, Fayed SA, Shalaby EA, El Shemy HA. *Syzygium cumini* (pomposia) active principles exhibit potent anticancer and antioxidant activities. Afr J Pharm Pharmacol 2011;5:948-56.

How to cite this article: Annadurai G, Masilla BR, Jothiramshekar S, Palanisami E, Puthiyapurayil S, Parida AK. Antimicrobial, antioxidant, anticancer activities of *Syzygium caryophyllatum* (L.) Alston. Int J Green Pharm 2012;6:285-8.

Source of Support: Nil, **Conflict of Interest:** None declared.