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# Chemical Composition, Antimicrobial, Antioxidant and Anticancer Activity of Leaves of *Syzygium benthamianum* (Wight ex Duthie) Gamble

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**Abstract:** The study was carried out on antimicrobial, antioxidant and anticancer activity of *Syzygium benthamianum* leaf extract. Chemical compositions of the leaf were analysed using GC/MS technique. A total of 24 compounds were identified among which 4-(4-ethylcyclohexyl)-1-pentyl-Cyclohexene (24.07 %) and Linoleic acid (15.16 %) are the major constituents. Antimicrobial activity of the leaf was observed on six bacterial and three fungal species, whose MIC values ranged from 100 to 500 µg/ml. At higher concentration, the extract exhibits higher scavenging activity (94.7 %) that was comparable with standard BHT. It was also observed that the leaf sample were able to effectively inhibit the growth of Hep 2 cells.

**Keywords:** *Syzygium benthamianum*, ethyl acetate extract, antimicrobial, antioxidant, anticancer activity.

## Introduction

*Syzygium* is a genus of flowering plants [1] belonging to family Myrtaceae comprising of about 1200 species, and spread across tropical Africa, subtropical and tropical Asia, Australia, New Caledonia, New Zealand, Pacific island; among these 80 species have been reported from China [2] and more than 75 species from India [3].

*Syzygium benthamianum* is one of the species that has been categorized as vulnerable tree species under the IUCN [4] red list of threatened species. *Syzygium* species have been reported to exhibit antidiabetic [5,6], antifungal [7,8], antiinflammatory [9], antibacterial [10], antioxidant [11], antihyperlipidemic [12] and growth inhibitory effects against oral pathogens [13]. *Syzygium* species are also found to posses antihyperglycemic activity [14], cytotoxic [15], antiangiogenic [15], Antinociceptive activity [16].

Till date there is no literature available on the medicinal properties of *Syzygium benthamianum*. Therefore, the present study was undertaken to analyze some of the medicinal properties of *Syzygium benthamianum* leaf extract constituents and their biological activities.

## Materials and methods

### *Plant material and extraction*

The plant material was collected from

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Kurichiarmala, Wayanad District in the state of Kerala, India, and identified as *Syzygium benthamianum* by Community Agrobiodiversity Centre (CAbC), M.S. Swaminathan Research Foundation, Wayanad, Kerala and voucher specimens deposited in the herbarium. The shade dried leaves were powdered and subjected to solvent extraction. Compound extraction was carried out using soxhlet apparatus [17] with ethyl acetate as solvent at 77°C for 4 hrs. Solvent was collected and evaporated using rotary evaporator at 77°C. The dried extracts were dissolved in dimethyl sulphoxide (DMSO) and evaluated for their efficacy against micro organisms.

**Gas chromatography-Mass spectrometry analysis**

The GC (Agilent 6890) conditions were as follows: DB-5 column (30 m X 0.25 mm X 0.25µm), injection in split mode (50:1), temperatures were maintained at 250°C in the injector and detector, helium was used as carrier gas at a flow rate of 1 ml/min; oven temperature was initially maintained at 100°C for 5 min and then raised to 220°C at a rate of 10°C/min and held at 220°C for 18 min. The MS (Agilent 5973 inert MSD) electron multiplier 2188.2 V, mass spectra data were acquired in the scan mode in m/z range 58-550. The compounds of the leaf extract were identified by comprising their retention indices (RI), with those on the stored in NIST (National Institute of Standards and Technology) library and by comparing their mass spectra with the data already available in literature [18].

**Microbial isolates**

Six Gram negative (*Pseudomonas aeroginosa, Proteus vulgaris, Proteus mirabilis, Vibrio cholera, Klebsiella pneumoniae* and *Escherishia coli*) and two Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) bacterial strains and three fungal (*Aspergillus niger, Alternaria alternata* and *Pencillium chrysogenum*) species were used in this study. The bacterial stock cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar medium, and were stored at 4°C.

**Determination of antimicrobial activity**

The extracts were tested for their antimicrobial activity by 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 h and 27°C for 48 h respectively. The test solutions of the dried extracts at the concentrations of 1000 µg, 500 µg, 250 µg, 100 µg/ml were impregnated on sterile discs. Streptomycin 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 h. The discs were placed on the surface of potato dextrose agar for fungi and incubated at 27°C for 48 h. Inhibition zones were calculated as the difference between disc diameter (6mm) and the diameters of inhibition [19]. The antibacterial activities were evaluated by the determination of minimum inhibitory concentration and minimum lethal concentration by micro broth dilution assay [20].

**Determination of antioxidant activity with 2,2 diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method**

The free radical scavenging activity of the sample was evaluated using DPPH [21]. The sample was prepared to the concentration of 1mg/ml using methanol. Pure methanol was taken as blank and 0.016 % butylated hydroxyl toluene (BHT) was taken as the standard. 2.7 ml of methanol, 100 µl of sample and 200 µl of DPPH reagent (1mg/ml) were added and these mixtures were kept in dark incubation at RT for 30 mins. Samples were visualized in UV-VIS spectrophotometer at wavelength of 517nm.

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 when the sample extract is added at a particular level and $A_{DPPH}$ is the absorbance of the DPPH solution [22].

**Anticancer activity: MTT Assay**

The 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. Cells were permitted to adhere for 24 h, and then treated with different dilution (1:1 to 1:64) of the extract for 24 h; 200 µl of MTT (5 mg/ml in PBS) were added to each well, and the cells were 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. The absorbance was recorded at the wavelength of 570 nm. The effect of the 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[24]:

Viability (%) = (Optical Density of sample x optical density of control) x 100

**Results and discussion**

Crude extract of leaf showed a total of 24 compounds among which 4-(4-ethyl-cyclohexyl)-1-pentyl-Cyclohexene (24.07 %) followed by Linoleic acid (15.16 %), 2,6,10,-14,18-Penta-methyl-2,6,10,14,18-eicosapen-taene (10.27 %), 9,17-Octadecadienal,(z)- (9.96 %), Z,E-3,13-Octadecadien-1-ol (7.14 %) and 7-Pentadecyne (7.36 %) are the major consti-tuents (Table 1).

**Table 1. Compound composition of *Syzygium benthamianum* leaves extract**

| Compound | RT [a] | RI [b] | % |
|---|---|---|---|
| Copaene | 7.363 | 1376 | 0.19 |
| α-trans-bergamotene | 7.820 | 1436 | 0.13 |
| (-)-isocaryophyllene | 8.007 | 1404 | 0.47 |
| *cis*-β-Farnesene | 8.324 | 1443 | 0.21 |
| α-Caryophyllene | 8.537 | 1454 | 0.17 |
| 1-Decene | 8.789 | 991 | 0.72 |
| α-selinene | 9.058 | 1494 | 0.16 |
| *cis*-α-Bisabolene | 9.232 | 1504 | 0.27 |
| Dodecyl acrylate | 12.283 | - | 0.44 |
| *cis*-Pinane | 14.817 | 983 | 4.45 |
| 1-Tridecyne | 15.273 | - | 0.80 |
| 5-Nonadecen-1-ol | 15.621 | - | 1.21 |
| (R)-(-)-14-Methyl-8-hexadecyn-1-ol | 19.228 | - | 0.65 |
| (-)-β-citronellene | 19.345 | - | 0.28 |
| 1,2-15,16-Diepoxyhexadecane | 19.441 | - | 0.22 |
| Linoleic acid | 24.743 | 2173 | 15.16 |
| Decahydro-8a-ethyl-1,1,4a,6-tetramethylnapthalene | 26.455 | - | 5.96 |
| Cyclohexene,4-(4-ethylcyclohexyl)-1-pentyl- | 28.711 | - | 24.07 |
| 7-Pentadecyne | 29.615 | - | 7.36 |
| 2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene | 29.710 | - | 10.27 |
| Z,E-3,13-Octadecadien-1-ol | 30.671 | - | 7.14 |
| 9,17-Octadecadienal,(z)- | 30.927 | - | 9.96 |
| Eicosane | 33.383 | 2000 | 4.22 |
| 3,6-Dimethyl-5-oxo-1,2,3,5-tetrahydroimidazol[1,2-a]pyrimidine | 34.122 | - | 5.00 |

[a]Compounds are listed and elution from DB5 column

[b]Values calculated from published literature (DB5 column)

The MIC values of the plant extract against the tested bacterial isolates ranged from 100 to 500 µg/ml. Plant extract at the concentration of 100 µg/ml inhibits the growth of *Proteus vulgaris* and *Proteus mirabilis* whereas, the growth of *Staphylococcus aureus* was inhibited at the concentration of 500 µg/ml. All other microbial species used in this study showed minimum inhibitory concentration at 250 µg/ml (Table 2).

The ethyl acetate extract of *Syzygium benthamianum* leaves 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). *Syzygium benthamianum* shows comparable scavenging activity with *Syzygium cumini* fruit [25] and *Syzygium aromaticum* buds [11].

MTT assay to evaluate the effect of the extract on cell viability of Hep2 cells was used. 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 benthamianum* showed higher activity on cancer cell lines and this result correlated with the activity exhibited by *Syzygium cumini* on AML cells [25].

Thus from the present study it was proven that the leaves of *Syzygium benthamianum* possess effective biological properties against pathogens and cancer cells. In addition also it exhibits high scavenging activity against free radical comparable with that of standard available drugs.

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**Table 2. Minimum Inhibition Concentration (MIC) of *Syzygium benthamianum* leaf extract against bacterial and fungal isolates**

| Bacterial and Fungal isolates | MIC µg/ml |
|---|---|
| *Escherichia coli* | 250 |
| *Pseudomonas aeruginosa* | 250 |
| *Klebsiella pneumonia* | 250 |
| *Proteus vulgaris* | 100 |
| *Staphylococcus aureus* | 500 |
| *Bacillus subtilis* | 250 |
| *Vibrio cholera* | 250 |
| *Proteus mirabilis* | 100 |
| *Alternaria alternata* | 250 |
| *Penicillium chrysogenum* | 250 |
| *Aspergillus* niger | 250 |

**Table 3. Antioxidant activity of *Syzygium benthamianum* leaf extract**

| S. No. | Sample | Concentration (µg/ml) | % Antioxidant activity |
|---|---|---|---|
| 1 | Blank | 0 | 0 |
| 2 | Standard | 400 | 95.7 ± 1.2 |
| 3 | Sample | 50 | 78.08 ± 1.65 |
| | | 100 | 85.7 ± 2.5 |
| | | 200 | 89.3 ± 3.1 |
| | | 400 | 94.7 ± 2.12 |

**Table 4. Anticancer activity of *Syzygium benthamianum* leaf extract**

| S.No. | Concentration (µg/ml) | Anticancer activity % |
|---|---|---|
| 1 | 50 | 50.75 |
| 2 | 100 | 57.64 |
| 3 | 250 | 68.15 |
| 4 | 500 | 75.44 |
| 5 | 1000 | 84.76 |

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