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# Chemical composition, antioxidant, anti-inflammatory and antitumor activities of *Eucalyptus globulus* Labill.

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*Eucalyptus globulus* L. is used in folk medicine throughout the world and its essential oils are widely used in modern pharmaceutical, food, and cosmetic industries. In this study, *E. globulus* leaves were extracted using three solvents (methanol, chloroform and hexane). The polyphenolics were quantified with HPLC and the volatiles analyzed by GC/MS with antioxidant, anti-inflammatory and antitumor activities. Results have shown a hierarchy of antioxidant and anti-inflammatory activity for three extracts as hexane > chloroform > methanol extracts with DPPH, FRAP, and oxygen radical absorbance capacity, respectively. A similar order of results was observed for antitumor activity by potato disc and colorimetric assays. The GC/MS analysis led to the identification of 1, 8-cineole (eucalyptol) as a major constituent of methanol (48.2%), chloroform (35.5%), and hexane (5.8%) extracts. Different phenolic acids (gallic acid, ellagic acid, syringic acid, and vanillic acid) and flavonoids (quercetin, rutin, and catechin) were highly abundant in methanol extract. The methanol extract of *E. globulus* exhibited the maximum antioxidant, anti-inflammatory, and antitumor activities. These results demonstrate *E. globulus* leaf extracts may be used as a potential source of bioactive compounds with remarkable antioxidant, anti-inflammatory, and antitumor activities.

**Keywords:** Eucalyptol, Flavonoids, Polyphenolics

Plants have an exclusive role in the health scenario since ancient time with medical and industrial importance[1]. For many years they have been utilized as tinctures, teas, infusions, decoctions, powders, and other herbal formulations from various countries like Arabian, American, Turkish, Chinese, Indian and many other traditions[2]. Realizing the wealth of medicinal flora, extracts from different parts of plants are widely evaluated for their biological activities including antioxidant, anti-inflammatory, and antitumor effects[3]. The bioactive compounds from plants, fruits, crops, and spices are important for many food industries because of their usefulness in various food preparations, drug development, formulations, and health promoting benefits[4]. Thus, the demand for natural compounds with biological value has increased due to the growing interest in the food and pharmaceutical industries by the development of the drugs which has fewer side effects and having potency against various diseases[5].

*Eucalyptus globulus* Labill. (Fam. Myrtaceae) is a fast growing plant species native to Australia and widely distributed worldwide. Eucalyptus has been used as folk medicine throughout the world, and the medicinal properties of these plants have been investigated[6]. Eucalyptus essential oils are widely used in modern pharmaceutical, food, and cosmetic industries and apart from its extensive use in the pulp industry, it also produces essential oils that are extracted on a commercial scale as raw materials in perfumery, cosmetics, food, beverages, aromatherapy, and phytotherapy[7,8]. Myrtaceae plant family is an important source of essential oils with a wide range of biological activities such as antibacterial, antifungal, analgesic, and anti-inflammatory properties[9]. The eucalyptol from *E. globulus* has been a component of natural sources of flavouring in many food and food products[10]. The application of eucalyptus essential oil/vapour used as food and beverages preservative with potent inhibitor of food spoilage yeasts not only *in vitro* but also in a real food system[11]. The essential oils from Eucalyptus species are among the most commonly traded essential oils in the world. Therefore, there is a growing interest in their usefulness as a natural additive for food, drugs, and both in scientific research and industrial production[12]. The essential oils from Eucalyptus can be found in the

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leaves of more than 300 species of this genus, and less than 20 of these have ever been exploited commercially for the production of essential oils rich in 1, 8-cineole by pharmaceutical and cosmetic industries[13].

As the chemical composition and biological activities of Eucalyptus extracts and essential oils have been a great concern in food and pharmaceutical industries we designed this study to analyze and evaluate the chemical composition, antioxidant, anti-inflammatory, and an antitumor activity of Eucalyptus leaves. Moreover, this study also includes a comparison of chemical constituents, polyphenolic compounds (phenolic acids and flavonoids), total phenolic contents and total flavonoid contents. In the current study a range of solvents [strongly polar (methanol), weakly polar (chloroform) and non-polar (hexane)] was used to evaluate the influence of polarity of extracting solvent in chemical composition and biological activities, so as to optimize the solvent for extraction and maximum biological activity.

## Materials and Methods

### Plant material

The leaves of *E. globulus* Labill were collected in April- May 2015 from Latur, Maharashtra, India (latitude 18° 24' N and longitude 76° 36' E**)** at 636 m above sea level. The plant was identified by Prof. S. N. Shinde (Botanist, Department of Botany, Rajarshi Shahu College, Latur, India). A voucher specimen was deposited in the same department.

### Chemicals

The chemicals gallic acid (GA), ellagic acid, ascorbic acid, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid, quercetin, thiobarbituric acid (TBA), trichloroacetic acid (TCA), nicotinamide adenine di-nucleotide phosphate (NADPH), and nitroblue tetrazolium (NBT) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Sodium carbonate, Folin–Ciocalteu's phenol reagent, ferric chloride, vincristine sulfate, DMSO, aluminum chloride, methanol, chloroform, and hexane were purchased from Sigma–Aldrich Co. (Seoul, Korea). Potassium ferricyanide, salicylic acid, heparin was purchased from Daejung Chemicals, Korea. The polyphenolic standards quercetin, catechin, rutin, luteolin, p-hydroxybenzoic acid, syringic acid, p-coumaric acid, and vanillic acid were procured from Sigma–Aldrich Co. (St. Louis, MO, USA).

### Preparation of plant extracts

Air-dried leaves of *E. globulus* were grounded using a food processor (Samsung model, R2N- SKU16968). The finely powdered 10 g of leaf powder was extracted with 100 mL methanol using a Soxhlet apparatus for 4 h and the resulting extracts were then centrifuged at 10,000 RPM for 10 min (Jisico Co., Ltd. Korea). The extract was then filtered through a 120-mm diameter filter paper. The extraction was repeated three times and the combined extracts were evaporated to dryness by rotary evaporation (Buchi Rotavapor, model V-500, Labotec, Essen, Germany). The crude concentrated extract was transferred to brown colored sample vials and stored at −20°C for further use. The similar extraction procedure was repeated with hexane and chloroform. The extracts thus produced by three different extraction solvents were analyzed independently for different assays[5].

### Gas chromatography/mass spectrograph (GC/MS) analysis

The GC/MS analysis of *E. globulus* leaf extracts (0.5 mg/mL) were carried out on a Shimadzu-2010 gas chromatograph, equipped with a FID data processor and a 60 m × 0.25 mm × 0.25 μm WCOT column coated with diethylene glycol (AB-Innowax 7031428, Japan). The GC–MS had electron energy of 70 Ev, ion source temperature of 230°C and electron emission of 34.6 μA. Helium was used as the carrier gas at a flow rate of 3.0 mL/min at a column pressure of 152 kPa. The temperature of the analyzer was maintained at 150°C. Helium was used as the carrier gas at a flow rate of 0.9 mL/min. The injector and interface temperature was set at 290 and 360°C, respectively. The oven temperature was programmed as 50°C (1 min) to 310°C (20 min), at an increasing rate of 6°C/min[4,8]. The percentage composition was calculated using the peak normalization method, assuming an equal detector response. The samples were further analyzed on the same Shimadzu instrument, fitted with the same column, and following the same temperature as above. Peak identification was carried out by comparison of the mass spectra with mass spectra available on a database of NIST05 and WILEY8 libraries. The compound identification was finally confirmed by comparison of their relative retention indices with literature values[14].

### HPLC analysis of polyphenolics

About 50 mg of the extracts (methanol, chloroform, and hexane) was dissolved in 20 mL of

distilled water, and 15 mL of this aqueous solution was hydrolyzed in a solution of 5 mL 6M HCl and 24 mL pure ethanol. After refluxing at 90°C for a specific time, the extract was cooled and made up to 50 mL, sonicated and analyzed. The final solution was filtered using 0.45 µm nylon membrane filter (Biotech, Germany) before high-performance liquid chromatography (HPLC) analysis. The contents of polyphenolics (phenolic acids and flavonoids) in hydrolyzed extracts were determined by high-performance liquid chromatography (HPLC). HPLC (Shimadzu, Japan) was performed using shim-pack CLC-ODS (C118); 25 cm × 4.6 mm, 5 µm column particle size was used. The mobile phase consisted of two solvents, (A) 2% phosphate buffer with pH 3; and (B) methanol. The method was isocratic for 5 min with 5% B, then changed with a linear gradient from 5 to 100% B over 30 min and for the last 5 min, it remained at 100% B. The flow rate was 0.7 mL/min and the detection was performed at 367 nm. The quantification was made with an external standard (quercetin, catechin, rutin, luteolin, gallic acid, ellagic acid, p-hydroxybenzoic acid, syringic acid, p-coumaric acid, and vanillic acid)[15,16].

**Total phenolic content**

Total phenolics in *E. globulus* leaf extracts (1 mM) were estimated using the Folin-Ciocalteu's colorimetric assay as described previously. Absorbance was measured at 760 nm by using UV-vis spectrophotometer (Shimadzu, Tokyo, Japan). The amount of total phenolic contents was calculated using gallic acid standard curve (05-100 µg/mL) and the content of phenolics expressed as µg gallic acid equivalents (GAEs) per mg of extract[17].

**Total flavonoid content**

For determination of total flavonoid content the methanol, chloroform and hexane extracts of *E. globulus* leaves (1 mM) were added to equal volumes of 2% $AlCl_3 6H_2O$ (2 g in 100 mL methanol) solution. The mixture was shaken vigorously and incubated for 10 min before the absorbance was read at 430 nm (Shimadzu, Tokyo, Japan). Total flavonoid contents in each sample were estimated using the standard curve for quercetin (1-50 µg/mL) and expressed as µg quercetin equivalents (QE) per mg of plant extract[18].

**DPPH radical-scavenging assay**

The DPPH-radical-scavenging potential of methanol, chloroform and hexane extracts of *E. globulus* leaves were examined by mixing with absolute ethanol and $1\times10^{-3}$ mol/L (0.18 mL) of DPPH. The obtained samples were properly mixed, incubated for 45 min and monitored at 515 nm against a blank using spectrophotometer (UV-Vis Shimadzu). All measurements were performed in triplicate. Quercetin (2 mM) was used as a standard. The radical scavenging activity was calculated using the formula: % inhibition = $(A_c (o) - A_A (t) / A_c (o) \times 100$ ($A_c$ (o) is the absorbance of control at t = 0 min and $A_A$ (t) is the absorbance of antioxidant at t = 1 h[17].

**FRAP assay (Reducing power assay)**

The $Fe^{3+}$ reducing the power of methanol, chloroform and hexane extracts of *E. globulus* leaves were determined using the previously described method. The reaction mixture contained 10 mL of *E. globulus* leaf extract in 3 mL of potassium ferricyanide solution (1 mM). The obtained mixture incubated at 50°C in a water bath for 20 min and 0.5 mL of trichloroacetic acid (TCA) (10%) was added to terminate the reaction. The upper portion of the solution (1 mL) was mixed with distilled water (1 mL) and 0.1 mL $FeCl_3$ solution (0.01%). The reaction mixture was incubated at room temperature for 10 min and the absorbance was measured using spectrophotometer at 700 nm using the blank solution. All tests were performed in triplicate. A higher absorbance of the reaction mixture indicated greater reducing power. Glutathione (2 mM) was used as a reference compound[19].

**OH-radical scavenging assay**

In this assay, the OH-radical was generated using the $Fe^{3+}$-ascorbate-EDTA-$H_2O_2$ system (the Fenton reaction). This reaction mixture contained, in a final volume 2-deoxy-2-ribose (1 mL, 2.8 mM), $KH_2PO_4$-KOH buffer (20 mM, pH 7.4), $FeCl_3$ (100 µM), EDTA (100 µM), $H_2O_2$ (1.0 mM), ascorbic acid (100 µM). To this, each test sample like 0.3 mL of *E. globulus* leaf extract (10 mg) was added. The obtained reaction with test samples then incubated for 1 h at 37°C, to which 1 mL 3% TCA, 1 mL of 2% aqueous TBA was added and incubated for 15 min at 90°C for colour development. After cooling, the absorbance was recorded at 532 nm using the blank solution. All tests were performed three times. Glutathione (2 mM) was used as a positive control. Percent inhibition was calculated by comparing the test and blank solutions[20].

**Anti-inflammatory activity**

For anti-inflammatory assays, 50 mg of *E. globulus* leaf extracts (methanol, chloroform, and hexane) were

used along with salicylic acid (SA), heparin (HE) and quercetin (QR) as standards.

*Diene-conjugate assay*

Conjugated diene assays for *E. globulus* extracts were performed using salicylic acid (1 mM) as a standard. The percent activity was calculated using the standard formula: % activity = (1 − T/C) × 100; where, T and C represent the absorbance of the test and control samples, respectively.

*β-Glucuronidase inhibition assay*

Inhibition of β-glucuronidase by *E. globulus* leaf extracts was determined using salicylic acid as a reference compound.

*Hyaluronidase activity inhibition*

The hyaluronidase inhibition by *E. globulus* leaf extracts were determined by modifying 96 well micro titer plate method. Heparin was used as a positive control.

*Lipoxidase activity inhibition*:

Inhibition of lipoxidase (LOX) by *E. globulus* leaf extracts was determined using quercetin as a reference compound. Percentage LOX inhibition was calculated using quercetin as a positive control[4,21].

**Antitumor activity**

Antitumor activity of *E. globulus* leaf extracts (methanol, chloroform, and hexane) were assessed with the potato disc method in which vincristine sulfate was used as positive and DMSO as a negative control. Percent inhibition of tumors was calculated using the formula: % inhibition= [(solvent control mean-tested extract mean) / solvent control mean] × 100. The $IC_{50}$ values were calculated by plotting percentage inhibition of tumors versus dose of extracts using regression analysis. All experiments were repeated three times[22].

**Statistical analysis**

The statistical values were presented as a mean ± standard deviation. One-way analysis of variance (ANOVA) for statistical analysis was used at 5% probability level.

## Results and Discussion

**Chemical composition of *E. globulus* leaf extracts**

GC/MS analysis of *E. globulus* leaf extracts revealed the identification and quantification of 40 essential oils (Table 1). Chemical investigation showed a significant difference in the composition of methanol, chloroform, and hexane extract. GC/MS analysis led to the identification of 1, 8-cineole (eucalyptol) as major constituent of methanol (48.2%), chloroform (35.5%) and hexane (5.8%) extracts. A considerable amount of eucalyptol was also reported in other *Euclayptus* species; *E. maidenii*, *E. nitens*, *E. dunnii*, and *E. grandis*[23,24]. Eucalyptol is an important component of aromatic plants and also known as 1, 8-cineol, 1, 8-cineole, eucalyptole, cineol and cineole; comprises up to 90% of the essential oil of some species of Eucalyptus[25]. Eucalyptol also reported in many medicinal and aromatic plants and evaluated for antioxidant, anti-inflammatory, antinociceptive and antifungal potential[24]. Furthermore, we examined that α-pinene, *cis*-verbenol, *α*-guaiene, *α*-terpineol acetate, and spathulenol were more common in methanol and chloroform extract, while aromadendrene, epiglobulol and chrysanthenone are more common in methanol and hexane extract and *o*-ocimene was only found in chloroform and hexene extracts, as shown in Table 1. Some constituents, such as β-sabinene, linalool, isoledene, ledene, epiglobulol, and cubenol had no literature data[23,24]. This possibly may be because the trees grown in different regions may exhibit differences in their chemical constituents.

**HPLC analysis**

The different (methanol, chloroform, and hexane) extraction yields (obtained after removal of the lipophilic fraction) were analyzed for identification and quantification of polyphenolic (phenolic acids and flavonoids) compounds in *E. globulus* leaf extracts by high-performance liquid chromatography (HPLC) and the corresponding polyphenolic content (μg/mL) of the analyzed extracts are shown in Table 2. Among the different extracts of *E. globulus* examined, methanol extract was most effective solvent for extraction of phenolic acids and flavonoids. Gallic acid (8.62±0.28 μg/mL) was examined as the major phenolic acid in the methanol extract of *E. globulus* followed by ellagic acid (6.58±0.12), vanillic acid (4.89±0.44) and *p*-hydroxybenzoic acid (4.36±0.14 μg/mL). Gallic acid (5.36±0.14) and ellagic acid (4.20±0.24 μg/mL) where the dominant phenolic acids in chloroform extracts while gallic acid (3.08±0.12) and *p*-hydroxybenzoic acid (2.10±0.05 μg/mL) are predominant in hexane extracts, respectively. All phenolic acids are observed in chloroform compared to methanol and hexane extracts; the p-coumaric acid (1.12±0.07 μg/mL) found as minor phenolic acids in the chloroform extract of *E. globulus*. Quercetin only

Table 1—Chemical composition (%) of methanol, chloroform and hexane leaf extracts of *E. globulus* analyzed by GC–MS

| Peaks | Compounds | Retention time (min) | Composition (%) | | |
|---|---|---|---|---|---|
| | | | Methanol | Chloroform | Hexane |
| 1 | *α*-Pinene | 4.65 | 5.2±0.3 | 8.8±0.6 | ND |
| 2 | Camphene | 5.14 | ND | 1.0±0.2 | ND |
| 3 | *β*-Pinene | 5.60 | ND | 0.8±0.03 | ND |
| 4 | *o*-Ocimene | 6.15 | ND | 2.1±0.4 | 1.2±0.2 |
| 5 | 1,8-Cineole | 7.14 | 48.2±1.2 | 35.5±2.5 | 5.8±0.6 |
| 6 | *cis*-Thujone | 8.40 | ND | 2.19±0.8 | ND |
| 7 | *cis*-Sabinol | 8.56 | 0.5±0.02 | ND | ND |
| 8 | β-sabinene | 9.12 | ND | 0.34±0.02 | ND |
| 9 | *α*-Campholenal | 9.40 | 0.46±0.03 | ND | ND |
| 10 | *cis*-Limonene oxide | 9.82 | ND | 0.28±0.01 | ND |
| 11 | Pinocarvone | 10.20 | ND | ND | 1.2±0.4 |
| 12 | limonene | 10.42 | 1.4±0.1 | ND | ND |
| 13 | *α*-Terpineol | 10.86 | ND | 0.98±0.03 | ND |
| 14 | γ-terpinene | 11.22 | ND | 0.28±0.01 | ND |
| 15 | Verbenone | 11.45 | 2.1±0.2 | ND | ND |
| 16 | Terpinolene | 12.30 | ND | ND | 0.28±0.03 |
| 17 | Carvone | 12.60 | ND | 0.65±0.02 | ND |
| 18 | Linalool | 13.5 | 0.36±0.04 | ND | ND |
| 19 | *cis*-Verbenol | 14.80 | 3.42±1.2 | 1.84±0.8 | ND |
| 20 | Chrysanthenone | 15.50 | 2.42±0.6 | ND | 1.38±0.2 |
| 21 | *α*-Guaiene | 18.48 | 6.52±1.3 | 4.21±0.9 | ND |
| 22 | Fenchol | 18.85 | ND | 0.18±0.02 | ND |
| 23 | *d*-Glucopyranose | 21.10 | 2.48±0.3 | ND | ND |
| 24 | α-terpineol acetate | 22.45 | 4.21±0.8 | 1.23±0.2 | ND |
| 25 | Globulol | 22.95 | 3.21±0.4 | ND | ND |
| 26 | 3-Heptadecene | 23.10 | ND | 1.86 ± 0.12 | ND |
| 27 | Isoledene | 23.25 | 0.22±0.04 | ND | ND |
| 28 | Spathulenol | 23.74 | 4.12±0.2 | 1.51±0.1 | ND |
| 29 | Isopulegol acetate | 23.95 | 0.20±0.01 | ND | ND |
| 30 | α-gurjunene | 24.45 | ND | 0.08±0.01 | ND |
| 31 | β-panasinsene | 24.95 | ND | 0.06±0.01 | ND |
| 32 | Aromadendrene | 25.60 | 8.74±1.4 | ND | 2.23±0.8 |
| 33 | Epiglobulol | 29.15 | 1.73±0.8 | ND | 2.88±1.2 |
| 34 | Cubenol | 29.30 | 0.84±0.02 | ND | ND |
| 35 | Ledol | 29.43 | 0.66±0.01 | ND | ND |
| 36 | α-cadinol | 30.30 | 1.24±0.4 | ND | ND |
| 37 | γ-eudesmol | 30.48 | ND | ND | 0.18±0.1 |
| 38 | Docosene | 31.16 | ND | 0.72±0.4 | ND |
| 39 | α-eudesmol | 31.25 | 0.98±0.04 | ND | ND |
| 40 | Palmitoyl chloride | 32.20 | ND | 2.14±0.8 | ND |

[Note: ND: Not detected, Compounds wee identified by comparison of retention time and mass spectra in NIST data. Data are represented as Mean ± SD of triplicate determinations]

observed in methanol (0.74±0.13 µg/mL) and chloroform (0.34±0.13 µg/mL) extracts and no any flavonoid found in hexane extract. The phenolics like catechin (0.52±0.21), rutin (0.32±0.09), and luteolin (0.22±0.04 µg/mL) were observed in methanol extract only. *E. globulus extracts* have similar to higher polyphenolic contents compared to *E. maidenii*, *E. grandis* and *E. urograndis* extracts, which is in agreement with a similar trend previously reported in the corresponding extracts. The results presented here

in this study confirm the occurrence of several polyphenolic compounds illustrated previous researchers for eucalyptus species[26,27].

**Total phenolics and flavonoid content**

The standard curve for total phenolic and flavonoid contents was expressed as µg gallic acid equivalents (GAEs) per mg and µg quercetin equivalents (QE) per mg of plant extract, respectively. Total phenolic contents of *E. globulus* leaf extracts were solvent dependent and ranged from 98.7±1.23, 156.3±2.65, and 242.5±4.32 µg GAE/mg of extracts in hexane > chloroform > methanol extracts, respectively (Table 3). Our results are in agreement with an earlier study in which extracting solvents exhibited the similar order (hexane > chloroform > methanol) for extraction of phenolic compounds [26]. The higher amount of phenolic compounds in the methanol extract might be attributed to its strong polar nature as most of the phenolic compounds are readily solubilized and majorly extracted by methanol[4]. Flavonoid contents of different *E. globulus* leaf extracts are mentioned in Table 3. The significant difference ($P$<0.05) was observed among flavonoid contents of methanol, chloroform and hexane extracts. The flavonoid contents varied from 52.5±1.6, 40.8±2.6, 24.6±1.3 µg QE/mg of extracts in hexane > chloroform > methanol extracts, respectively (Table 3).

**Antioxidant activity**

The results for DPPH radical scavenging (%DPPH), reducing ability (%RA), and OH radical scavenging (%OH) activity of different (methanol, chloroform, and hexane) *E. globulus* leaf extracts were summarized in Table 4. All three extracts under study were effective towards the scavenging of DPPH radicals and exhibited the antioxidant capacity in the range of 26.6-72.6%. The maximum antioxidant potency was found in methanol (72.6±0.4) compared to chloroform (60.5±0.3) and hexane (56.8±0.2) extracts; towards the stabilization of DPPH radicals and methanol (58.7±0.4) compared to chloroform (46.4±0.5) and hexane (34.5±0.3) extracts; towards the ferric reducing power, while methanol (38.5±1.2) compared to chloroform (32.2±1.1) and hexane (26.6±0.9) extracts by OH radical scavenging activity. The DPPH radical scavenging assay has often been performed for evaluation of the antiradical activity of antioxidants since DPPH possesses an odd electron responsible for giving a strong absorption peak at 515 nm[25,26]. In the light of the structure-activity relationship, it seems that both DPPH radical scavenging activity and reducing potential are related to the degree of hydroxylation, which may be

Table 2—Polyphenolic compounds (µg/mL) in leaf extract of *E. globulus*

| Polyphenolics | *E. globulus* leaf extracts | | |
|---|---|---|---|
| | Methanol | Chloroform | Hexane |
| Quercetin | 0.74±0.13 | 0.34±0.13 | ND |
| Catechin | 0.52±0.21 | ND | ND |
| Rutin | 0.32±0.09 | ND | ND |
| Luteolin | 0.22±0.04 | ND | ND |
| Gallic acid | 8.62±0.28 | 5.36±0.14 | 3.08±0.12 |
| Ellagic acid | 6.58±0.12 | 4.20±0.24 | 0.86±0.09 |
| *p*-hydroxybenzoic acid | 4.36±0.14 | 3.55±0.08 | 2.10±0.05 |
| Syringic acid | 3.86±0.48 | 2.45±0.01 | 1.24±0.09 |
| *p*-coumaric acid | ND | 1.12±0.07 | ND |
| Vanillic acid | 4.89±0.44 | 2.56±0.11 | ND |

[Note: ND: Not detected. Values are Mean ± SD of triplicate determinations]

Table 4—Antioxidant activities tested for methanol, chloroform and hexane extracts of *E. globulus* leaf extracts.

| Extracts/Compounds | $IC_{50}$±SD (extracts/fractions[µg/mL]; bioactive components [mM]) | | |
|---|---|---|---|
| | DPPH | FRAP | $H_2O_2$ |
| Methanol | 72.6±0.4 | 58.7±0.4 | 38.5±1.2 |
| Chloroform | 60.5±0.3 | 46.4±0.5 | 32.2±1.1 |
| Hexane | 56.3±0.6 | 34.5±0.3 | 26.6±0.9 |
| Quercetin | 65.8±0.2 | ND | ND |
| Glutathione | ND | 46.8±0.1 | 41.5±0.5 |

[ND: Not Determined; Values are mean ± SD (n = 3)]

Table 3—Total phenolic contents, total flavonoid contents and antitumor activity of *E. globulus* leaf extracts and tested positive controls

| *E. globulus* and positive controls | Total phenolics (µg GAE/mg) | Total flavonoids (µg QE/mg) | Percent (%) death (*A. tumefaciens*) | Antitumor activity $IC_{50}$ (µg/mL) |
|---|---|---|---|---|
| Methanol | 242.5±4.32 | 52.5±1.6 | 82.6±1.32 | 65.2±1.48 |
| Chloroform | 156.3±2.65 | 40.8±2.6 | 75.2±1.20 | 122.3±2.11 |
| Hexane | 98.7±1.23 | 24.6±1.3 | 62.8±0.98 | 188.1±1.68 |
| Vincristine sulphate | NT | NT | 98.8 ±1.80 | 11.5±0.09 |

[NT: Not tested, All data are represented as Mean ± SD (n = 3)]

involved in donating electrons and thereby stabilizing the radical compounds[18,19]. The reaction rate constant for OH radicals is extremely high, wherein they reacting indiscriminately almost every type of biomolecules within the cell and might deviate the normal physiological functions of the cells[20,21]. OH radicals have been reported to be a key player in the physiological regulation and control of cell functions, and also it has been reported that in many inflammatory disorders; such as rheumatic arthritis, gout and the reaction of nitric oxide with superoxide generates peroxynitrite, which, under the acid conditions often found in regions of inflammation and ischemia, yields the hydroxyl radicals[19,21].

**Anti-inflammatory activity**

Anti-inflammatory activities *E. globulus* leaf extracts were determined using diene-conjugate, β-glucuronidase, lipoxidase, and hyaluronidase in the vitro inhibition assay. This study revealed the significant anti-inflammatory activity of the all tested extracts (methanol, chloroform, and hexane). Methanol extract exhibited the significant anti-inflammatory activity as compared to all samples tested (chloroform and hexane) (Table 5). At 25 mg, methanol extract inhibited all enzymes more strongly than did the remaining samples (61.2% for β-glucuronidase, 34.2% for diene-conjugate, 50% for hyaluronidase, and 46.9% for lipoxidase, respectively). Several lines of evidence have been provided, mostly in different animal models of inflammation, and to a much lesser extent in humans or human cells, of the effectiveness of either *E. globulus* leaf extracts or of compounds isolated there from against inflammation and its mediators[28]. The β-glucuronidase mainly occurs in lysosomes of neutrophils and plays an important role as mediators in the initiation and progression of inflammation[4]. The hydroperoxides (diene-conjugates) generation is one of the intermediate steps in membrane lipid peroxidation[17]. The lipid peroxidation phenomenon plays a vital role in many inflammatory disorders. The lipid peroxidation results in oxidative modifications of the apoprotein which is mainly involved in macrophage uptake and atherogenesis[28]. The result indicates that the *E. globulus* leaf extracts may reduce the lipid peroxidation by their antioxidant and anti-inflammatory activities.

**Antitumor activity**

The inhibition of *Agrobacterium tumefaciens* induced tumors (or Crown Gall) in potato disc tissue is an assay based on antimitotic activity and have been used to detect a broad range of known and novel antitumor agents[15]. Crown Gall is a neoplastic plant disease caused by *A. tumefaciens* and the validity of this bioassay is predicated on the observation that certain tumorigenic mechanisms are similar in plant and animal[27]. Successful crown gall formation on a potato disc surface during an antitumor study is dependent on viable *A. tumefaciens*. It became necessary to avoid the lethal concentrations against *A. tumefaciens*[17]. Our results showed that methanol, chloroform and hexane extracts of *E. globulus* leaf more prominent to induce death of neoplastic disease causing agent (*A. tumefaciens*) in plants These three extracts reveled effects on the viability of *A. tumefaciens* and exhibited considerable antitumor activity with $IC_{50}$ values of 65.2, 122.3 and 188.1 µg/mL for methanol, chloroform and hexane extracts, respectively (Table 3). However, our results showed that the extent of tumor inhibition by an extract depends on the strain being used for the assay. The antitumor activity of methanol extract was high with $IC_{50}$ values of 82.6 µg/mL, whereas, the antitumor activity of chloroform extracts against the viability of *A. tumefaciens*, with maximum percent death of 75.2 µg/mL. Poor antitumor activity was examined

Table 5—Anti-inflammatory activities tested for methanol, chloroform and hexane extracts of *E. globulus* leaf extracts.

| Extracts/Compounds | Inhibition (%) (Mean±S.D from three experiments) | | | |
|---|---|---|---|---|
| | β-glucuronidase | Diene-conjugate | Hyaluronidase | Lipoxidase |
| Methanol | 61.2±1.2 | 34.2±0.3 | 50.2±2.1 | 78.9±1.5 |
| Chloroform | 52.4±1.1 | 28.4±0.1 | 42.1±1.0 | 64.2±1.3 |
| Hexane | 45.1±1.2 | 22.1±0.2 | 32.6±1.4 | 55.5±1.1 |
| Salicylic acid (SA) | 78.6±2.1 | 48.5±0.5 | ND | ND |
| Heparin (HE) | ND | ND | 65.5±1.2 | ND |
| Quercetin (QR) | ND | ND | ND | 85.2±1.9 |

[ND: Not Determined; Values are mean ± SD (n = 3)]

in hexane extract against *A. tumefaciens*, with $IC_{50}$ values of 62.8 µg/mL. Overall, the antitumor activity of methanol extract was significantly ($P < 0.05$) higher than chloroform and hexane extracts against *A. tumefaciens*. The potential antitumor activity of methanol extract may also be endorsed to the in it. The difference in tumorogenic approach of methanol, chloroform and hexane extracts towards the *A. tumefaciens* might be attributed to the distinctive profile of the high amount of total phenolics and total flavonoid and bioactive essential oil contents (Tables 1 and 2) and their innovative mechanism of action.

## Conclusion

The data suggested that the methanol, chloroform and hexane extracts of *Eucalyptus globulus* leaves might be used as therapeutic agent against oxidative stress, inflammation, and tumor formation, but further *in vivo* and clinical studies should be required for *E. globulus* leaf proactive compound isolation and characterization as a drug. Methanol extract raveled maximum amount of total phenolic and total flavonoid with potent biological activities compared to chloroform and hexane; which showed moderate to poor effects for scavenging of free radical, This study provides a scientific support towards the medicinal use of *E. globulus* suggesting that increasing the intake extracts and utilization of their active compounds may be helpful in preventing or reducing the progress of some lifestyle related diseases. Although it is not simple to extrapolate from *in vitro* results, the diversity of the pharmacological activities of *E. globulus* leaf observed in this study suggests that extracts of this *E. globulus* species may be of value for application in pharmaceutical industry and human health.

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## References

1 Nile SH & Park SW, HPTLC densitometry method for simultaneous determination of flavonoids in selected medicinal plants. *Front Life Sci*, 8 (2015a) 97.

2 Jaiswal Y, Liang Z & Zhao Z, Botanical drugs in ayurveda and traditional chinese medicine. *J Ethnopharmacol*, 194 (2016) 245.

3 Al-Rifai A, Aqel A, Al-Warhi T, Wabaidur SM, Al-Othman ZA & Badjah-Hadj-Ahmed AY, Antibacterial, antioxidant activity of ethanolic plant extracts of some *Convolvulus* species and their dart-tof-ms profiling. *J Evid Based Complementary Altern Med*, 2017 (2017) 5694305.

4 Nile SH & Park SW, Chromatographic analysis antioxidant anti-inflammatory and xanthine oxidase inhibitory activities of ginger extracts and its reference compounds. *Ind Crops Prod*, 70 (2015b) 238.

5 Yeh HY, Chuang CH, Chen HC, Wan CJ, Chen TL & Lin LY, Bioactive components analysis of two various gingers (*Zingiber officinale* Roscoe) and antioxidant effect of ginger extracts. *LWT- Food Sci Technol*, 55 (2014) 329.

6 Takahashi Y, Sugimoto K, Soejima Y, Kumagai A, Koeda T, & Shojo T, Inhibitory effects of Eucalyptus and Banaba leaf extracts on nonalcoholic steatohepatitis induced by a high-fructose/high-glucose diet in rats. *Biomed Res Int*, (2015) 296.

7 Ali B, Al-Wabel NA, Shams S, Ahamad A, Khan SA & Anwar F, Essential oils used in aromatherapy: a systemic review. *Asian Pac J Trop Biomed*, 5 (2015) 601.

8 Khan Y, Ansari HR, Chauhan RR, Tamboli ET & Ahmad S, Comparative gas chromatography-mass spectroscopy, Fourier transform infrared spectroscopy, and high-performance liquid chromatography analysis of essential oils extracted using 4 methods from the leaves of *Eucalyptus globulus* L. *Drug Develop Ther*, 7(2016) 81.

9 Cascaes MM, Guilhon GMSP, Andrade EHA, Zoghbi MGB & Santos LS, Constituents and pharmacological activities of Myrcia (Myrtaceae): a review of an aromatic and medicinal group of plants. *Int J Mol Sci*, 16 (2015) 23881.

10 Vargasa R, Kowalski V & Vecchiettia A, Fermentable sugars from *Eucalyptus globulus*: Process optimization. *Comput Chem Eng*, 93 (2016) 343.

11 Tyagi AK, Bukvicki D, Gottardi D, Tabanelli G, Montanari C & Malik A, Eucalyptus essential oil as a natural food preservative: *in vivo* and *in vitro* antiyeast potential, *Biomed Res Int*, 2014 (2014) 969143.

12 Neiva D, Fernandes L, Araújo S, Lourenço A, Gominho J, Simões R & Pereira H, Chemical composition and kraft pulping potential of 12 eucalypt species, *Ind Crops Prod*, 66 (2015) 89.

13 Ishnava KB, Chauhan JB & Barad MB, Anticariogenic and phytochemical evaluation of *Eucalyptus globulus* Labill. *Saudi J Biol Sci*, 20 (2013) 69.

14 Abd-Elkhalek A & Heikal M, Variation in the essential oil content and its composition in *Eucalyptus cinerea* leaves and its relation to some environmental factors. *J Essent Oil Bearing Plants*, 20 (2017) 995.

15 Ashraf A, Sarfraz RA, & Mahmood A, Phenolic compounds characterization of *Artemisia rutifolia* spreng from Pakistani flora and their relationships with antioxidant and antimicrobial attributes. *Int J Food Prop*, 20 (2017) 2538.

16 Murai Y & Iwashina T, Phenolic Compounds from *Sanguisorba obtusa* Endemic to Japan. *Bull Natl Mus Nat Sci Ser B*, 42 (2016) 43.

17 Nile SH & Park SW, HPTLC analysis antioxidant anti-inflammatory and antiproliferative activities of *Arisaema tortuosum* tuber extract. *Pharmceut Biol*, 52 (2014a) 221.

18 Nile SH, Nile AS, & Keum YS, Total phenolics, antioxidant, antitumor, and enzyme inhibitory activity of Indian medicinal and aromatic plants extracted with different extraction methods. *3 Biotech*, 7 (2017) 76.

19 Nile SH & Park SW, Antioxidant α-glucosidase and xanthine oxidase inhibitory activity of bioactive compounds from maize (*Zea mays* L). *Chem Biol Drug Des*, 83 (2014b) 119.

20 Wu P, Ma G, Li N, Deng Q, Yin Y & Huang R, Investigation of *in vitro* and *in vivo* antioxidant activities of flavonoids rich extract from the berries of *Rhodomyrtus tomentosa* (Ait.) Hassk. *Food Chem*, 173 (2015) 194.

21 Nile SH, Keum YS, Nile AS, Jalde SS & Patel RV, Antioxidant, anti-inflammatory, and enzyme inhibitory activity of natural plant flavonoids and their synthesized derivatives, *J Biochem Mol Toxicol*, 32 (2018) e22002.

22 Ashraf A, Sarfraz RA, Rashid MA & Shahid M, Antioxidant, antimicrobial, antitumor, and cytotoxic activities of an important medicinal plant (*Euphorbia royleana*) from Pakistan. *J Food Drug Anal*, 23 (2015) 109.

23 Barbosa LCA, Filomeno CA & Teixeira RR, Chemical variability and biological activities of Eucalyptus spp. essential oils. *Molecules*, 21 (2016) 1671.

24 Dhakad A K, Pandey VV, Beg S, Rawat JM & Singh A, Biological, medicinal and toxicological significance of *Eucalyptus* leaf essential oil: A review. *J Sci Food Agric*, 98 (2018) 833.

25 Miranda I, Lima L, Quilhó T, Knapic S, Pereira H, The bark of *Eucalyptus sideroxylon* as a source of phenolic extracts with anti-oxidant properties. *Ind Crops Prod*, 82 (2016) 81.

26 Boulekbache-Makhlof L, Slimani S & Madani K, Total phenolic content antioxidant and antibacterial activities of fruits of *Eucalyptus globulus* cultivated in Algeria. *Ind Crops Prod*, 41 (2013) 85.

27 Lantz RC, Chen GJ, Sarihan M, Solyom AM, Jolad SD & Timmermann BN, The effect of extracts from ginger rhizome on inflammatory mediator production. *Phytomedicine*, 14 (2007) 123.

28 Nile SH, Venkidasamy B & Keum YS, Characterization of organic acids, sugars, antioxidant and anti-inflammatory activities of *Citrus reticulate* Blanco cv. Nagpur. *Indian J Exp Biol*, 55 (2017) 604.