Comparative Phytochemical Studies and Evaluation of Radical Scavenging Activity in Selected *Jasminum* Species

*1Sulaiman C.T., 2Soudha V., 1Deepak M., 1Indira Balachandran*

1 *Phytochemistry Division, Centre for Medicinal Plants Research, Arya Vaidya Sala, Kottakkal*
2 *Department of Chemistry, Farook College, Calicut*

**ABSTRACT**

Phytochemical studies were carried out in five Jasminum species. The chemical profiles of different species were compared using chromatographic techniques such as TLC and HPLC. The total phenolic content (TPC) and radical scavenging capacity were also evaluated. The DPPH EC₅₀ value was found least for *Jasminum Grandiflorum* (7.5µg) showing its highest antioxidant activity. The EC₅₀ (DPPH) values vary as, 15 µg (*J.angustifolium*), 12 µg (*J.auriculatum*), and 7.5µg (*J. grandiflorum*).

**Key words:** TLC, HPLC, DPPH, Total phenolics.

**INTRODUCTION**

Medicinal plants and plant-derived medicines are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemical (Van Wyk and Michael Wink, 2009). Nearly all cultures from ancient times have used plants as a source of medicine. The World Health Organization (WHO) has listed 21,000 plants worldwide, reported to have medicinal uses. India is the largest producer of medicinal herbs and is called the *botanical garden of the world* (Trivedi, 2007).

*Jasminum* genus with about 200 species belonging to family Oleaceae are of three types: shrub or bush form, vines and trees, native to tropical and warm temperate regions. Many *jasminum* plants prominently feature white, yellow or pink flowers with sweet fragrance and are unscented *jasminum* species is used to treat many conditions such as amenorrhea, leprosy, skin diseases and also as an analgesic, antidepressant and anti-inflammatory (http://www.ehow.com/about_6325794_jasmine-plants). *Jasminum grandiflorum* is a scrambling sub erect twining evergreen shrub (Anonymous, 2004), native to India, France, Italy, China, Japan, Morocco and Egypt [Chopra et al, 1958].The leaves are opposite, entire ovate to somewhat elliptic in shape with acuminate mucronate apex, whereas flowers are terminal and axillary cymes, calyx lobes are long, linear (Cooke, 1967 & Nadkarni, 1976). Roots are useful in cephalalgia, mental debility, chronic constipation, flatulence, strangury, sterility, dysmenorrhea, amenorrhoea, ringworm, leprosy, skin diseases and giddiness. *Jasminum sambac* is commercially grown in India, Thailand, China and Philippines. It is an evergreen vine or shrub reaching up to 1-3 m. The leaves are ovate; phyllotaxy is opposite or in whorls of three. The flowers blooms throughout the year and are produced in clusters of 3-12 together. The plant traditionally used as an analgesic, antidepressant, anti inflammatory, antiseptic, aphrodisiac, sedative, expectorant and tonic (uterine) effects. Roots are used to treat wounds and snake bites. The leaves and flowers have antipyretic and decongestant properties. Phytochemical studies shown that the roots contains drotiacontanoxic acid, drotiacontanol, oleanolic acid, daucosterol and hesperidin (Zhang et al, 2004) and leaves contain sambacosides A, E and F (Tanahashi, 1988) flower contains molihuasiade A-E, sambaeoside A (Zhang et al, 1995).

*Jasminum angustifolium*, distributed in south India (kerala, Karnataka) on the hills of lower elevation (Bown, 1995) Leaves are simple ovate-lanceolate, acute, glabrous and flowers are either solitary or more usually in three. Petals are linear, obtuse and acute Hepatoprotective effect of ethanolic and chloroform extract of *Jasminum angustifolium* were evaluated against carbon tetrachloride (1ml/kg) induced hepatic damage and was evidenced by reduction in level of alkaline phosphatase (ALP), alkaline amino transferase (ALT), aspartate amino transferase (AST), cholesterol, glucose, total protein and bilirubin concentration in blood (Joshi et al, 2008). *Jasminum auriculatum* grows almost throughout South India, on dry slopes of the Western Ghats. The roots are useful in skin diseases especially for ringworm and flowers are fragrant, bitter, acrid, sweet, refrigerant, astringent, cardiothoictic, diuretic and depurative in nature. They are useful in burning sensation, hyperdesia, ulcers, odontalgia, stomatopathy, opthalmopathy, cardiacopy, urolithiasis, nephrolithiasis, strangury and dermatopathy (Ghosh, 1984). *Jasminum auriculatum* leaves have been reported to contain lupeol and jasminol (Deshpande et al, 1967). Alcoholic and aqueous extracts of flowers of *Jasminum auriculatum* showed diuretic activity by increasing the total volume of urine and concentrations of potassium and sodium salts in urine and antiurolithiatic...
activity by reducing the elevated urinary oxalate synthesis. (Bahuguna et al., 2009).

MATERIALS AND METHODS
Extraction: Plant materials (leaf) were collected from Herb Garden, Arya Vaidya Sala- Kottakkal and authenticated by Taxonomy division of Centre for Medicinal Plant Research, Arya Vaidya Sala, Kottakkal, Kerala. The powdered samples (2g each) were subjected to sequential extraction using 100ml of petroleum ether, chloroform, and methanol respectively for 5 hours. The final volume is made up to 50 ml to get 40-mg/ml solution. These solutions are taken for different analyses. TLC Analysis: TLC analysis of petroleum ether and chloroform extracts was carried out on a precoated silica plate (F254 Merck) using toluene: ethyl acetate as mobile phase in the ratio 9:1. TLC analysis of methanol extracts was carried out using toluene: ethyl acetate as mobile
phase in the ratio 8:2. The plates were developed up to 9 cm and visualized under UV 254 nm, UV 366 nm and in visible light after derivetizing with Anisaldehyde-sulphuric acid (ANS) reagent.

HPLC Analysis: HPLC profiling was done using a Shimadzu High Performance Liquid Chromatographic system equipped with LC-10ATVP pump, SPD M10AVP Photo Diode Array Detector in combination with CLASS-VP 6.12 SP5 integration software. The mobile phase used for the separation was HPLC grade 0.1% formic acid in Acetonitrile (A) and methanol (B) in a time programming 0-10 10% A, 10-20 30% A, 20-30 50% A, 30-40 60% A and 40- 50 70 % A. The column used was C18 – ODS (Octadecylsilane), Lichrosphe r RP 18e (5μm) (Merck) with a Phenomenex guard column (4mm x 2 mm i.d: 5µm). The samples were injected using a 20 µl loop (Rheodyne Rohnet Park, CA, USA). The flow rate was maintained to 0.75 ml/min.

Total Phenolic Content (TPC): The assay was based on the reduction of phosphomolybdate ion of Folin-Ciocalteu reagent by the phenolate ion of sample. A desired amount of plant extract, distilled water and 1 N Folin-Ciocalteu reagent was taken into a tube and mixed thoroughly. After an interval of 3 min, 2 ml of 2% sodium carbonate solution was added and the mixture was allowed to stand for 30 min with intermittent shaking. The absorbance of the mixture was measured at 550 nm using spectrophotometer (Shimadzu, Japan). Different Gallic acid standards (2, 5, 7, 10, and 15 μg/ml) were used for obtaining a standard curve (Singleton et al., 1965). The total phenolic content was expressed as Gallic acid equivalents (GAE) per gram of sample.

DPPH Assay: DPPH radical scavenging assay DPPH radical scavenging activity of the leaf extracts were determined according to the method described by Kukic et al., . First, 4.0 mL of test material at different concentrations were reacted with 0.50 mL of 1.0 mM DPPH solution and kept in the dark for 30 minutes, following which the absorbance was measured at 517 nm against a blank sample consisting of 4.0 mL of MeOH and 0.50 mL DPPH solution. DPPH scavenging activity was calculated using the equation:

% DPPH radical scavenging activity = \( \frac{(A_o-A_s)}{A_o} \times 100 \).

Where \( A_o \) is the absorbance of the blank sample, and \( A_s \) is the absorbance of the test material. The IC_{50} value represented the concentration of test material that caused
50% scavenging activity. Catechin was used as positive controls.

RESULTS AND DISCUSSION
TLC Analysis: The thin layer chromatographic profiles of different extracts revealed the chemical pattern of each extract. On visualizing under 254 nm a compound with $R_f$ 0.54 was found in both petroleum ether and chloroform extracts of all the species. A band with $R_f$ 0.37 is present in petroleum ether extracts of *J. flexile*, *J. grandiflorum* and *J. sambac*, while the same is absent in *J. angustifolium* and *J. auriculatum*. The band with $R_f$ 0.12 is present in the petroleum ether extracts of *J. auriculatum*, *J. flexile* and *J. sambac*. At $R_f$ 0.85 an intense band is seen in the petroleum ether extract of *J. grandiflorum*. This particular compound is specific for *J. grandiflorum* (Fig: 1.1).

On visualizing under 366 nm compounds with $R_f$ 0.37, 0.49, 0.55 are present in all species of petroleum ether extracts and chloroform extracts. A compound with $R_f$ 0.91 is specific for the petroleum ether extract of *J. grandiflorum*. A compound with $R_f$ 0.87 is present in petroleum ether extracts of *Jasminum Angustifolium*, *J. flexile* and *J. sambac*. (Fig: 1.2) For chloroform extracts, a common band was observed at $R_f$ 0.80. The compounds with $R_f$ 0.58, and 0.77 are specific for *J. angustifolium* (Figure 1.2).

After derivatization with Anisaldehyde–Sulphuric acid (ANS) a compound with $R_f$ 0.55 was present in both...
petroleum ether and chloroform extracts of all the species. A compound at Rf 0.15 is found in both the petroleum ether extract and chloroform extract of J. auriculatum and J. grandiflorum. In the case of petroleum ether extracts a band at Rf 0.23 is found in all species except J. grandiflorum and a band at Rf 0.69 is found in J. auriculatum, J. flexile and J. sambac. A band with Rf 0.36 is present in all species of petroleum extracts (Figure 1.3).

For methanol extracts, on visualizing under 254 nm, it was found that a compound with Rf 0.83 is present in all species. At Rf 0.11 a compound is present in J. angustifolium and J. grandiflorum and at Rf 0.17 a compound is present only in J. auriculatum (Figure 1.4). On visualizing under 366 nm a pink colour band is present with Rf 0.04 in all species. A band at Rf 0.10 is found in J. angustifolium, J. grandiflorum and J. sambac. A compound with Rf 0.18 is present only in J. auriculatum. Compounds with Rf 0.71, 0.78 and 0.83 are found in all species except J. auriculatum (Figure 1.4).

Total Phenolic Content (TPC): A comparative determination of Total Phenolic Content (TPC) of methanol extracts of leaves of the five species was determined by Folin-ciocalteu phenol reagent using 2. standard Gallic acid. The amounts of total phenolic content were expressed as mg Gallic acid Equivalents (GAE) per gram. It was found that the phenolic content is highest in Jasminum Grandiflorum (51.875mg GAE). The phenolics content of J. flexile (8.125 mg GAE) and J. sambac (10.625 mg GAE) are comparatively less (Figure 4.1.6)

DPPH Assay: The DPPH EC_{50} value was found least for Jasminum Grandiflorum (7.5μg) showing its highest 5. antioxidant activity. The EC_{50} (DPPH) values vary as, 15 μg (J.angustifolium), 12 μg (J.auriculatum), and 7.5μg (J. grandiflorum). It was found that a positive correlation between total phenolic content and radical scavenging activity. J. flexile & J. sambac do not have radical 7. scavenging activity.

HPLC Analysis: HPLC Analysis of methanol extracts was done for the comparison of their chemical profiles. In 8. the chromatogram of J. angustifolium major peaks were found at Rf 2.603, 3.541, 3.904, 4.992 and 5.195. J. auriculatum showed major peaks at Rf 1.899, 2.272, 9. 2.411, 2.891 and 6.443. The peaks at Rf 1.920, 2.293 and 3.008 are the major peaks in J. flexile. For J. 10. grandiflorum, the major peaks obtained at Rf 2.155, 2.699, 3.733,4.160,4.779 and 5.035. The chromatogram of J. sambac showed peaks at Rf 1.984, 2.731, 3.723, 4.181 and 5.035. The compound corresponds to Rf 1.9 was found in all species except J. grandiflorum with different area percentage showing the quantitative variation. Except J. flexile peak with Rf 3.7 is common in all other species. A 12. peak at Rf 2.2 was observed for J. auriculatum & J. flexile with area percentage 30.825 % and 73.040 % respectively indicating the higher quantity of that particular component. The species J grandiflorum & J. sambac showed a compound with Rf 5.03 having area % more than 60. This compound is also present in J. flexile, but in small quantity. J. grandiflorum and J. sambac also showed peaks at Rf 7.1 and 10.0.

J. angustifolium and J. auriculatum, showed two peaks (Rf 5.1 & 6.1) as major compounds. Two common peaks with Rf 6.5 & 7.3 were found in both J. angustifolium and J. flexile. A compound with Rf 6.9 was observed in J. angustifolium & J. sambac only as traces. A compound with Rf 7.584 was found in J. auriculatum, J. flexile and J. sambac.

The present studies are of highly valuable as it is developed a standard method for the comparative phytochemical analysis such as Thin layer Chromatography, High pressure Liquid Chromatography for the identification of major Phytoconstituents present in different extracts of the selected species. The evaluation of radical scavenging activity with respect to the phenolic compounds present in each species is a major outcome of this present study.

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