GC-MS analysis of methanolic extract of *Litsea decanensis* gamble and its free radical scavenging activity

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**ABSTRACT**

There is an increasing interest in the antioxidant effect of compounds derived from plants, which could be relevant in relation to their nutritional incidence and their role in health and disease. The aim of the present study is to develop a rapid method for the quantitative determination of organic compounds in herbs by GC-MS technique. *Litsea* is one of the most diverse genera of evergreen or deciduous trees or shrubs belonging to the Laurel family, Lauraceae. Most *Litsea* plants contain alkaloids, flavonoids, terpenes, lactones and volatile oil constituents. *Litsea* plants exhibit a variety of biological activities including antimicrobial, hypothermic and anti-tumor activities. The methanolic extracts of *Litsea decanensis* extract was tested by GC-MS for its phytochemical constituents was found to be quassin, squalene, stigmasterol, oleic acid and vitamin E are the major constituents present in the extract. The total phenolic and total flavonoid contents also were measured. Antioxidant activity of methanolic extract was also studied.

**Key words:** *Litsea*, Phytochemicals, Antioxidant, Reducing Power.

**INTRODUCTION**

There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease.[1] In the recent years, the interest for the study of the organic compounds from plants and their activity has increased. A lot of extraction methods and analytical methods as spectrophotometry, high performance liquid chromatography, capillary electrophoresis (HPLC), gas chromatography – flame ionization detection (GC-FID), gas chromatography–mass spectrometry (GC-MS) are developed for the study about plant active compounds.[2] The combination of an ideal separation technique (GC) with the best identification technique (MS) made GC–MS an ideal technique for qualitative and quantitative analysis for volatile and semi-volatile compounds. The aim of the present study is to develop a rapid method for the quantitative determination of organic compounds in herbs by GC-MS technique.

*Litsea* plants exhibit a variety of biological activities, including antimicrobial, hypothermic and anti-tumor activities.[3] It has been reported the aphrodisiac activity ethanolic extract of the bark of *Litsea chinensis*.[4] The methanolic extract of *L. cubeba* and its fractions showed remarkable antioxidant activity in comparison with α-tocopherol and ascorbic acid.[5] There are no previous reports on the phytochemical information on *Litsea decanensis*. Hence the plant extract was tested by GC-MS for its phytochemical constituents. The anti-oxidant activity, total phenolic and total flavonoid contents of the plant extract were also studied.

**MATERIALS AND METHODS**

**GC-MS description:**

For the GC-MS analysis a 30×0.25mm×1µmdf a 100% Dimethyl poly siloxane column; was used in Clarus 500 Perkin Elmer gas chromatograph with a Turbo mass gold-Perkin Elmer detector. The samples were introduced via an all-glass injector working in the split mode, with Helium as the carrier gas, linear velocity 32 cm/s. Temperature program: 110°C-2 min hold, upto 200°C at the rate of 10° C/min-no hold, Up to 280°C at the rate of 5° C/ min-9 min hold. The mass range scanned was 45-450 g, and total MS running time is 36 min. The identification of components was accomplished using library NIST version- year 2005.

**Plant material and extraction:**

Ascorbic acid, Folin-Ciocalteu reagent (Himedia), Rutin (Merck), Gallic acid is purchased from Sigma Chemical Co. All the solvents and chemicals used were analytical grade, obtained from Merck India.

Aerial parts of *Litsea decanensis* were collected during the month of January from forest regions of Chittoor district in Andhra Pradesh. The plant material was authenticated by Department of Botany, Sri Venkateshwara University, Tirupathi. A voucher specimen has been deposited in the Department of Pharmacognosy, Jayamukhi College of Pharmacy.

The aerial parts of the plant material were air dried to dryness at room temperature and under shade, and then powered to a fine grade by using a laboratory scale mill. The shade dried parts of the plant were powered which was kept in air tight plastic bag until use. The powder defattigated with petroleum ether and the resulting marc was then extracted with methanol and 0.1% Hydrochloric acid. The extract was then filtered, dried and then used.

**Tests for total phenols:**

The content of total phenolic compound in plant methanolic extracts was determined by the method of Folin-Ciocalteu.[6] For the preparation of calibration curve 1 ml aliquots of 0.024, 0.075, 0.105 and 0.3 mg/ml ethanolic Gallic acid solution were mixed with 5 ml Folin-Ciocalteu reagent (diluted ten-fold) and 4 ml (75 g/L) sodium carbonate. The absorption was read after 30 min at 20°C at 765 nm and the calibration curve was drawn. One ml aqueous plant extract (10g/L) was mixed with same reagents as described above, and after 1 h the absorption was measured for the determination of plant phenolics. All determinations were performed in triplicate. Total content of phenolic compounds in plant methanol extracts in Gallic acid equivalents (GAE) was calculated by the following formula

\[ C = \frac{c \times V}{m} \]

Where, 
C-Total content of phenolic compounds, mg/g plant extract, in GAE, 
c-Concentration of Gallic acid established from the calibration curve, mg/ml 
V-Volume of the extract

**Test for total flavonoids:**

The content of flavonoids was determined according to colorimetric method as described by zou et al.[7] In brief, 0.5 ml of sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% NaNO₂ solution. After 6 min of incubation, 0.15 ml of 10% AlCl₃ solution was added and then allowed to stand for 6 min, followed by adding 2 ml of 4% NaOH solution to the mixture. Immediately water was added to the sample to bring for another 15 min. The mixture absorbance was determined at wavelength 510 nm. The total flavonoid content was expressed in milligrams of rutin equivalents per gram of extract. The amount of flavonoids in plant extracts in rutin equivalents (RE) was calculated by following formula

\[ X = \frac{(A_m - A_o)}{A_m} \]

Where: 
X — flavonoid content, mg/g plant extract in RE; 
A_o — the absorption


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of plant extract solution, m — the weight of rutin in the solution; g; \(A_p\) — the absorption of standard rutin solution; g — the weight of plant extract g.

**Determination of free radical scavenging activity by DPPH radical scavenging activity:**

The free radical scavenging activities of the extracts on the extracts were measured in vitro by 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) assay. A 0.1ml of 0.1mM/l DPPH radical solution was mixed with 3ml of various concentrations of extract (50, 100, 300µg/ml) dissolving in methanol. The absorbance was measured at517 nm after 30 min of reaction. The percent scavenging of the sample was calculated according to the equation.

\[
\% \text{ Scavenging} = \left(1 - \frac{A_s}{A_o}\right) \times 100
\]

Where, \(A_s\) — Absorbance of control, \(A_o\) — Absorbance of sample, Ascorbic acid, Gallic acid, and used as control.

**Assay of reducing power:**

The total reducing power was determined according to method Oyaizu. Briefly, 1ml of different concentrations of our methanolic extracts (100, 200 & 300 µg/ml in distilled water) were mixed with phosphate buffer (2.5 ml, 0.2 M, pH=6.6) and potassium ferricyanide [KFe(CN)₆] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and FeCl₃ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

**RESULTS & DISCUSSION**

**GC-MS analysis:**

The results of GC-MS analysis of *Litsea decanensis* extracts were given in Table 1. Individual flavonoids were identified from RT, mass data, and by comparison of the data of the standard compounds with those in the library and literature. Some compounds remained unidentified due to the lack of reference substances and library spectra. We have identified some of the compounds using NIST05.

**Antioxidant activity:**

The scavenging effects of our extracts on DPPH radical are shown in the Figure 2. Maximum scavenging activity was found at concentration of 200µg/ml while the minimum scavenging activity was found at 25µg/ml.

**Total phenolic analysis:**

The total phenolic content present in the methanolic extract of *Litsea decanensis* is 0.2 mg/ml Gallic Acid equivalent.

**Total flavonoid analysis:**

The total flavonoid content of methanolic extract of *Litsea decanensis* is 2.26 mg/g plant extract in Rutin equivalents.

**Reducing power analysis**

The reductive capabilities of the plant extract compared to rutin as shown in the Figure 3. The reducing power of extract of *Litsea decanensis* was very potent and the reducing power of the extract was increased with quantity of sample. The plant extract could reduce the most Fe³⁺ ions, which had a lesser reductive activity than the standard of rutin.

Free radicals play a crucial role in the development of tissue damage in pathological events. Our rapid and simple method outlined the active principles in the studied herbs which are responsible for some therapeutic effects. The extraction method presented is simple, rapid and inexpensive, with reduced solvent consumption, GC–MS method used for the analysis of the obtained extracts can be an interesting tool for testing the amount of some active principles in herbs used in cosmetic, drugs, pharmaceutical or food industry. The acidic fractions were silylated and subjected to GC-MS investigation. It is evident from this table that all fractions have a complex chemical composition. Some of the GC-MS peaks remained unidentified, because of lack of authentic samples and library data of corresponding compounds. The extract is a complex mixture of many constituents including 11 identified compounds. All the eleven compounds have been identified for the first time in *Litsea decanensis* and two of them are flavonoids and four of them are phenolic acids.

5-Hydroxymethyl-2-furancarboxaldehyde (C-1) converts Hydroxy methyl furfural (HMF) to 2, 5-dimethylfuran (DMF) which is a liquid bio-fuel in certain ways which is superior to ethanol. It also helps in the production of plastics and it is mainly used in the treatment of sickle cell anemia. Vitamin E (C-2) is the key compound involved in many physiological processes, such as neurological and immune functions. The most common role of vitamin E is its antioxidant effect, protecting molecules and tissues from deleterious free radicals. Biosynthesis of various biologically active steroids necessary for the normal vital activity of plants starts from phytosterols, including stigmastanol. Linolenic acid (C-3) is a poly unsaturated fatty acid it is used in the formation of prostaglandins. Oleic acid (C-4) is a fatty acid used to lower blood levels of cholesterol and oils used in food industry. Stigmasterol (C-5) is used as a precursor in the manufacture of synthetic progesterone, a valuable human hormone that plays an important physiological role in the regulatory and tissue rebuilding mechanisms related to estrogen effects, as well as acting as an intermediate in the biosynthesis of androgens, estrogens, and corticoids. It is also used as the precursor of vitamin D3. It also possesses potent antioxidant, hypoglycemic and thyroid inhibiting properties. Squalene (C-6) has been proposed to be an important part of the Mediterranean diet as it may be a chemo-preventative substance that protects people from cancer. Squalene is used in cosmetics, and more recently as an immunologic adjuvant in vaccines. Quassin (C-7) is a white bitter, crystalline substance found in nature with a bitter threshold of 0.08 ppm and it is 50 times bitterer than quinine.

It has been reported that reactive oxygen species contribute to various pathological conditions and endogenous defense mechanisms have evolved to offer protection in these conditions. An increase in the antioxidant reserves of the organism can reduce oxidative stress. Many synthetic antioxidant components have shown toxic and/or mutagenic effect, which have shifted the attention towards the naturally occurring antioxidants. Phenols are very important plant constituents there is a highly positive relationship between total phenols and antioxidant activity of many plant species, because of the scavenging ability of their hydroxyl groups. It was also reported that phenolic compounds are effective hydrogen donors, making them very good antioxidants.

*Litsea decanensis* seems to have highest concentration of polyphenolic compounds therefore it is showing antioxidant activity and reducing activity. So the present study was conducted to investigate the antioxidant potential of *Litsea decanensis*. Antioxidant activity of *Litsea decanensis* extract has been revealed *in-vitro* by DPPH radical scavenging activity. There are numerous methods for evaluating the antioxidant activity of both natural and artificial compounds. The method using stable DPPH radical however, is a widely used one because of its simplicity and requiring relatively short time compared to other methods. Possible mechanism of DPPH scavenging was suggested to be through reduction of this radical by antioxidant compound to a more stable DPPH form. Because of its unpaired electron DPPH has an absorption maxima at 520 nm and as it gets reduced (eg; as this electron becomes paired off) in the presence of free radical scavengers the absorbance decreases stoichiometrically with respect to the number of electrons taken up.

For the measurement of the reducing ability, Fe³⁺-Fe²⁺ transformations in the presence of phenolic fraction of *Litsea decanensis* was found. The reducing ability of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing ability of extract was compared with Rutin. The reducing power of extract of *Litsea decanensis* was found to increase with rising concentrations.

**Table 1: List of Phytochemical constituents identified by GC-MS**

<table>
<thead>
<tr>
<th>SNo</th>
<th>RT</th>
<th>Name of the Compound</th>
<th>Molecular formula</th>
<th>MW</th>
<th>Peak Area%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.96</td>
<td>4H-Pyrain-4-one,2,3,5,6-tetrahydro-3,6-dihydroxy-6-methyl-</td>
<td>C₇H₁₂O₅</td>
<td>188</td>
<td>288</td>
</tr>
<tr>
<td>2</td>
<td>7.23</td>
<td>2-Fluorcarboxaldehyde,5-(hydroxymethyl)-</td>
<td>C₆H₇NO</td>
<td>144</td>
<td>0.72</td>
</tr>
<tr>
<td>3</td>
<td>10.51</td>
<td>1,6-Anhydro-6-D-glucopyranose (levoglucosan)</td>
<td>C₁₀H₁₄O₄</td>
<td>278</td>
<td>58.87</td>
</tr>
<tr>
<td>4</td>
<td>16.49</td>
<td>8-Hexadecanoic acid</td>
<td>C₁₈H₃₄O₂</td>
<td>278</td>
<td>58.87</td>
</tr>
<tr>
<td>5</td>
<td>18.97</td>
<td>Vitamin E</td>
<td>C₂₂H₃₄O₂</td>
<td>278</td>
<td>58.87</td>
</tr>
<tr>
<td>6</td>
<td>19.71</td>
<td>9,12,15-Octadecatrienonic acid, (3Z,6Z,9Z)</td>
<td>C₂₆H₄₀O₂</td>
<td>278</td>
<td>58.87</td>
</tr>
<tr>
<td>7</td>
<td>19.54</td>
<td>Oleic Acid</td>
<td>C₁₇H₃₀O₂</td>
<td>192</td>
<td>19.54</td>
</tr>
<tr>
<td>8</td>
<td>25.13</td>
<td>1,2-Benzeneedicarbonyl acid, diisooctyl ester</td>
<td>C₃₀H₄₂O₂</td>
<td>430</td>
<td>5.44</td>
</tr>
<tr>
<td>9</td>
<td>28.87</td>
<td>Squalene</td>
<td>C₃₀H₄₄O₂</td>
<td>430</td>
<td>5.44</td>
</tr>
<tr>
<td>10</td>
<td>29.50</td>
<td>Quassin</td>
<td>C₂₇H₃₂O₂</td>
<td>278</td>
<td>58.87</td>
</tr>
<tr>
<td>11</td>
<td>31.14</td>
<td>Stigmasterol</td>
<td>C₂₅H₄₄O₂</td>
<td>388</td>
<td>45.87</td>
</tr>
</tbody>
</table>
CONCLUSION
GC-MS analysis of *Litsea decanensis* extract showed a high complexity profile, containing approximately 11 components, mainly quassin, squalene, stigmasterol, vitamin E and oleic acid. These compounds are having multiple pharmacological activities. In the present study we have demonstrated that *Litsea decanensis* extract possess potent antioxidant and free radical scavenging activities, and reducing abilities of which could be derived from such as flavonoids and phenolic acids. This study may be useful to explore the pharmacological activity of the extract and individual phytochemicals present in this extract.

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REFERENCES

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