EVALUATION OF BIOACTIVE COMPOUNDS PRESENT IN PIPER BETLE LINN. BY ELUTION CHROMATOGRAPHY COUPLING TECHNIQUE

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ABSTRACT

The present study investigates the presence of various phytochemicals in the methanolic leaf extract of Piper betle and further analysis of the bioactive compounds present in it by elution chromatography coupling technique. The preliminary qualitative phytochemical screening revealed the existence of alkaloids, flavonoids, carbohydrate, proteins, phenols, saponin, phytosterols and terpenoids. Totally 25 bioactive phytochemical compounds were identified from P. betle by GC – MS analysis. This study forms a basis for the biological characterization and phyto-pharmaceutical significance of the identified compounds. The presence of various bioactive compounds justifies the usage of betel leaf as an herbal choice for treating various diseases.

KEYWORDS: Piper betle, GC-MS analysis, Phytochemical Screening, Phytoconstituent, medicinal plants, Elution chromatography coupling technique

INTRODUCTION

Plants are very rich source of secondary metabolites with varied structural arrangements and interesting biological activities.[1] The Indian subcontinent is one of the richest countries in terms of plant genetic resources. As per the statement of World Health Organisation (WHO), more than 80% of the population in developing countries still depends on the traditional medicine for their primary healthcare needs.[2] For centuries, people rely on different medicinal plant extracts and formulations and trying to alleviate and treat various diseases.[3] Moreover, the medicinal plants are safe, effective, cheaper, easily accessible and with no or lesser incidence of side effects when compared to the conventional medicine. Natural
products play a significant role in the discovery and novel drug development for the treatment and prevention of diseases.\[4-6\] Growing awareness in herbal products has today accelerated the demand and growth of medicinal plant-based industries.\[7\]

The genus piper belongs to the piperaceae family, which comprise of more than 1000 species. Piper betle Linn. a dioecious, perennial creeper, climbing by many small adventitious rootlets, grows to a height of about one metre, widely cultivated in hotter and damper parts of the country.\[8,9\] Leaves are simple, alternate, ovate, cordate, acuminate or acute, entire and bright green in colour. Male spikes of this plant are dense and cylindrical while female spikes are pendulous. This is indigenous to South and South East Asia\[10,11\] and widely found in damp forests. It is cultivated in India, Srilanka, Bangladesh, Burma and Nepal.\[12,13\] In the Indian state of Tamilnadu, three varieties of Piper betle leaves viz., Sirugamani, Karpoori and Vellaikodi are accessible mostly.\[14\]

Experiments have shown that betel leaf possess wide range of therapeutical activity such as antibacterial, anticariogenic, antifungal, antilarval, antiprotozoal, antifilarial, antihistaminic, antidiabetic, antiinflammatory, hepatoprotective, antiulcer, antifertility, cardioprotective, antihyperlipidaemic, antiplatelet, vasodilatory and immunomodulatory effects.\[12\] It is called as betel leaf, wild pepper and betel pepper etc., Asia leads to reflect the religious diversity of the planet. Betel leaves are considered most auspicious and are widely used during the religious functions in Asia even today. The study of the bioactive principles present in the plant is very essential because most of the drugs were synthesized followed by a careful study of their constituents and structures.\[15\]

Elution chromatography coupling technique\[16\] is the chromatographic elution technique such as gas chromatography (GC), liquid chromatography (LC) or capillary electrophoresis (CE) which is coupled with the mass spectrometer (MS) to analyse a complex mixture. The separated products must be introduced into the spectrometer in the gaseous state for GC/MS while in the liquid state for LC/MS and CE/MS. GC – MS is an analytical hyphenated technique which combines the features of gas-liquid chromatography and mass spectrometry for the elution, detection and quantification of different substances in the complex mixture of the test sample.

Major application of GC-MS analytical technique includes drug detection in biological fluids, investigation of explosives, fire investigation, environmental analysis, forensic trace evidence
and well-proven recognition of unknown samples. GC-MS analysis was also carried out to recognize the name, molecular weight and structure of the compounds of the test samples. The unknown organic compounds in the complex mixture can be determined by interpretation and matching the spectra with reference spectra.[17] Keeping this in sight, the present study has been carried out to explore the phyto-constituents present in the methanolic leaf extract of *P. betle* L.

**MATERIALS AND METHODS**

**Collection and Identification of *Piper betle* L.**
Leaves of *Piper betle* L. were collected from Kolli Hills, Namakkal District, South India. Plant material was identified by examination of its morphological characteristics, authenticated and voucher specimen was deposited in Rapinet Herbarium, St. Joseph’s College, Tiruchy -620 002, Tamil Nadu, India.

**Preparation of the leaf extract**
Freshly collected leaves of *Piper betle* L. were shade dried and size reduced to powder with the use of mechanical grinder. 10 grams of the pulverized material were soaked in 100 mL of methanol and kept on a rotary shaker for 24 hrs. The extract was then filtered through Whatman No. 1 filter paper and the process was repeated till the extraction of all soluble compounds. The extract was concentrated in a rotary evaporator under reduced pressure. The dried material was collected and stored at -20°C for further experimental procedures.

**Qualitative Phytochemical Screening**
Plant extract was subjected to the preliminary qualitative phytochemical screening methods described by various authors[18-25] for identification of its phytochemicals. The plant extract was screened for the presence of pharmacologically active compounds such as alkaloids, flavonoids, carbohydrates, phytosterols, proteins, phenolics, terpenoids and saponins.

**GC-MS Spectral analysis**[26]
20 g of the powdered leaf sample was soaked in 75 ml of methanol for 24 hrs. The filtrates were collected by evaporation under liquid nitrogen. The GC-MS analysis was carried out using a Clarus 500 Perkin-Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass Gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl polysiloxane), 300 m x 0.25 mm x 1 x m df capillary column. The instrument was set at initial temperature of 110°C and maintained for 2 min.
After this period, temperature of the oven was raised up to 280°C, at the rate of an increase of 5°C/min and maintained for 9 min. Temperature of injection port was ensured as 250°C and helium flow rate as 1.0 ml/min. The electron impact ionization voltage was 70 eV. The samples were injected in split mode as ratio 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of the mass spectra were compared with authentic standard stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The molar percentage composition of each component was calculated from relative peak area of each component in the chromatogram.

RESULTS AND DISCUSSION

Phytochemical Analysis

The present study made an attempt to screen for its phytochemicals. The preliminary phytochemical screening of methanolic leaf extract of Piper betle showed the maximum number of secondary metabolites such as alkaloids, flavonoids, carbohydrates, proteins, phenols, saponins, phytosterols and terpenoids (Table-1). These secondary metabolites especially alkaloids, flavonoids and phenolic compounds were formerly reported with various pharmacological activities.[27,28] This qualitative preliminary phytochemical screening is used as an important key step for subsequent determination of phytochemicals for their therapeutical activity like antioxidant, cardioprotective, anticancer, antiinflammatory, antimicrobial, etc.

Table-1: Preliminary phytochemical screening of methanolic leaf extract of P. betle L.,

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>PHYTOCONSTITUENTS</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>02</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>03</td>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>04</td>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>05</td>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>06</td>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>07</td>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>08</td>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>09</td>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Phlobotannins</td>
<td>-</td>
</tr>
</tbody>
</table>

(+ ) Presence of phytoconstituents, (- ) Absence of phytoconstituents.
Presence of flavonoids, phenols and alkaloids exposed that the species may be used as analgesic, antispasmodic, antimicrobial, anticancer, anti-inflammatory and antioxidant. Some flavonoids such as quercetin and rutin are well known for their anti-inflammatory, antihistaminic and antiviral activities. Alkaloids has been known for its anti-hypertensive and detoxifying properties.

**GC-MS analysis of *Piper betle* L.**

Phytochemical compounds present in the methanolic leaf extract of *P. betle* was identified by GC-MS analysis. Totally 25 peaks were identified, where all phytoconstituents were characterized and identified (Table-2).

### Table 2: Qualitative and quantitative determination of biochemical constituents in *Piper betle* by GC-MS.

<table>
<thead>
<tr>
<th>Peak</th>
<th>Peak name</th>
<th>Chemical Formula</th>
<th>Mol. Weight</th>
<th>Retention time</th>
<th>Peak Area</th>
<th>% Peak Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>R-(-)-1,2-propanediol</td>
<td>C₃H₆O₂</td>
<td>76</td>
<td>3.39</td>
<td>245444</td>
<td>0.3733</td>
</tr>
<tr>
<td>2.</td>
<td>Methyl acetoxyacetate</td>
<td>C₅H₈O₄</td>
<td>132</td>
<td>4.05</td>
<td>693807</td>
<td>1.0551</td>
</tr>
<tr>
<td>3.</td>
<td>Ethanamine, N-ethyl-N-[(1-methylethoxy)methyl]-</td>
<td>C₈H₁₉NO</td>
<td>145</td>
<td>6.29</td>
<td>463061</td>
<td>0.7042</td>
</tr>
<tr>
<td>4.</td>
<td>1,3,5-Triazine-2,4,6-triamine</td>
<td>C₅H₁₁NO</td>
<td>101</td>
<td>9.69</td>
<td>724979</td>
<td>1.1025</td>
</tr>
<tr>
<td>5.</td>
<td>Piperidine, 1-hydroxy-</td>
<td>C₅H₁₁NO</td>
<td>101</td>
<td>10.62</td>
<td>108790</td>
<td>0.1654</td>
</tr>
<tr>
<td>6.</td>
<td>3-Amino-2-oxazolidinone</td>
<td>C₃H₆O₂N₂</td>
<td>102</td>
<td>10.80</td>
<td>115456</td>
<td>0.1756</td>
</tr>
<tr>
<td>7.</td>
<td>4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-</td>
<td>C₆H₈O₄</td>
<td>144</td>
<td>10.91</td>
<td>1091318</td>
<td>1.6597</td>
</tr>
<tr>
<td>8.</td>
<td>Butanedioic acid, ethyl-, dimethyl ester</td>
<td>C₈H₁₄O₄</td>
<td>174</td>
<td>11.23</td>
<td>292630</td>
<td>0.4450</td>
</tr>
<tr>
<td>9.</td>
<td>Phenol, 2,3,4,6-tetramethyl-</td>
<td>C₁₀H₁₄O</td>
<td>150</td>
<td>13.90</td>
<td>198301</td>
<td>0.3016</td>
</tr>
<tr>
<td>11.</td>
<td>Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-</td>
<td>C₁₅H₂₄</td>
<td>204</td>
<td>15.74</td>
<td>191904</td>
<td>0.2918</td>
</tr>
<tr>
<td>12.</td>
<td>à-Cubebene</td>
<td>C₁₅H₂₄</td>
<td>204</td>
<td>16.74</td>
<td>27986</td>
<td>0.0426</td>
</tr>
<tr>
<td>13.</td>
<td>2,5-Dimethoxybenzoic acid</td>
<td>C₉H₁₀O₄</td>
<td>182</td>
<td>16.89</td>
<td>43332268</td>
<td>65.8992</td>
</tr>
</tbody>
</table>
The high percentage of 2,5-Dimethoxybenzoic acid (65.8992%), Phenol, 2-methoxy-3-(2-propenyl)- (6.1550%), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (4.9169%), Phytol (8.0181%), Piperine (1.6883%) compounds were identified. The GC-MS chromatogram of *P. betle* is shown in Figure 1. In general, the isolated high peak compounds were reported to possess antibacterial, larvicidal and antifungal properties.
CONCLUSION

The results reveal that the methanolic leaf extract of P. betle have a number of bioactive phytoconstituents, which are responsible for numerous therapeutic activities. The compounds identified by GC-MS in methanolic leaf extract of P. betle are medicinally valuable and possess a wide variety of pharmacological applications. Further isolation and testing of phytoconstituents for their individual biological activity will undoubtedly bring promising results in the discovery and development of novel drugs from mother nature.

REFERENCES


Figure – 1: GC - MS spectra of methanolic leaf extract of Piper betle L.


