QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF ETHANOLIC LEAF EXTRACTS OF *CAMELLIA SINENSIS* (TEA) AND *VICA FABA* (BROAD BEAN)

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Abstract:
Medicinal plants contains various bioactive compounds involve in physiological activity against many pathogenic diseases. Aim of the present study is to determine the qualitative and quantitative phytochemical constituents of ethanol leaf extract of *Camellia sinensis* and *Vicia faba*. Quantitative analyses of extracts were analyzed using GC-MS reveals presence of major active compounds. The active compounds were identified by comparing their retention time and peak area. Many compounds were used in industry for various applications like flavor, antioxidant, anti-inflammatory, antimicrobial, pesticide and cancer preventive.

Index terms: Phytochemical, GC-MS, *Camellia sinensis*, *Vicia faba*

I Introduction:
Medicinal plants play a vital role in the production of new drug in India. Several steps have been taken to promote plant medicine and to integrate them into clinical practice[1]. Medicinal plants are considered as rich source of ingredients that could be used in drug development and synthesis. Medicinal plants also play a critical role in the development of human cultures around the world[2]. The bioactive compounds in the traditional medicines and fruits are important to protecting a number of diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world depends on traditional medicine for their primary healthcare. Medicinal plants contain rich source of secondary metabolites with interesting biological activities. These secondary metabolites are with a variety of structural arrangements and properties[3]. Gas chromatography has a very wide field of applications. But, its first and main area of use is in the separation and analysis of multi component mixtures. It act as the analytical technique for quality control and phytotherapeutics. *Camellia sinensis* is the commercially available crop cultivated throughout South Asia. This plant is a member of theaeace family which is an evergreen shrub with large number of branches. The tea leaf has much beneficial medicinal value on the body. In the recent herbal era, green tea is one of best tonic for healthy being of life. Black tea leaves can also effective against oxidative stress[4]. Fava bean is cultivating for 5,000 years, one of the oldest legumes. It is cultivated along Central Asia and Mediterranean region. According to the United Nations Food and Agriculture Organization (FAO), 60% of the total production of Fava bean is produced by China[5].

II Materials and Methods:
2.1 Collection of plant materials:
The leaves of *Camellia sinensis* and *Vicia faba* were collected from the region of Nilgiri District. The collected leaves were washed and cleaned with distilled water and shade dried for one week.
2.2 Solvents for extraction:
Ethanol was the solvents chosen for the present study. The solvents used were of analytical grade.

2.3 Preparation of plant extract:
The dried Camellia sinensis and Vicia faba leaf samples were crushed to coarse powder and 5g of powdered sample was taken and suspended in 50ml of chosen solvent Ethanol at the concentration of 90%. The mixture was kept in the shaker with continuous stirring for 4 days at 32ºC. The extracts were then filtered using sterile Whatmann filter paper (No.#1). The filtrates obtained were kept open at room temperature for solvent evaporation. The semi solid sample extract were stored in refrigerator for further analysis[6][7].

III QUALITATIVE PHYTOCHEMICAL ANALYSIS:
Preliminary phytochemical analysis were made with sample extracts in order to detect the presence of various types of phytoconstituents like alkaloids, carbohydrates, flavonoids, glycosides, phenolic compounds, proteins, aminoacids, phytosterols, saponins, tannins and terpenoids[7]. The extract was diluted with its respective solvent and used for photochemical analysis.

3.1 DETECTION OF ALKALOIDS:
Test for Alkaloids were performed as follows[8].
Wagner’s test:
0.1ml of sample extract was treated with Wagner’s reagent (Iodine in potassium iodide).

3.2 DETECTION OF CARBOHYDRATES:
Fehling’s test:
0.1ml of the extract were hydrolysed with 0.5ml of dilute HCl, neutralised with 0.5ml of alkali and added 4-5 drops of Fehling’s solutions and heated in a boiling water bath for 5minutes[8].

3.3 DETECTION OF FLAVONOIDS:
Alkaline reagent Test:
1.0ml of extracts were treated with 10% of sodium hydroxide solution resulted in the increase with the intensity of yellow colour. The colour was later decreased by addition of few drops of dilute Hydrochloric acid.

3.4 DETECTION OF GLYCOSIDES:
Modified Borntranger’s test:
To 2.0ml of extracts added 2.0ml of 10% ferric chloride solution and heated in boiling water bath for about 5minutes. The mixture was cooled and treated with 4.0ml of benzene. The benzene layer was separated and diluted with ammonia solution[8].

3.5 DETECTION OF PHENOLS:
Lead Acetate Test:
To 2.0 ml of the extracts, 3.0 ml of 10% lead acetate solution were added[7].

3.6 DETECTION OF PROTEINS:
Xanthoproteic test:
3.0 ml of the extracts were treated with few drops of concentrated nitric acid.

3.7 DETECTION OF AMINOACIDS:
Ninhydrin test:
To 3ml of the extracts, 3dops of 0.25% ninhydrin reagent was added and boiled for few minutes.

3.8 DETECTION OF PHYTOSTEROLS:
Libermann Burchard’s test:
2.0 ml of the extracts were treated with 2.0 ml of chloroform and filtered. The filtrates were treated with 1.0 ml of acetic anhydride, boiled and cooled. 2 drops of concentrated sulphuric acid was added[9].

3.9 DETECTION OF SAPONINS:
Froth Test:
Extracts were diluted with 5.0 ml of distilled water and this was shaken in a graduated cylinder for 15 minutes[8].

3.10 DETECTION OF TANNINS:
Ferric Chloride Test:
To 2.0 ml of the extracts, 1.0ml of neutral 5% ferric chloride solution were added .

3.11 DETECTION OF TERPENOIDS:
Salkowski Test:
0.5ml of extracts were treated with 1.0ml of chloroform and 1.0ml of concentrated sulphuric acid[9].

IV QUANTITATIVE ANALYSIS:
GC-MS:
Gas Chromatography (GC) and Mass Spectrometry (MS), is used to analyze complex organic and biochemical mixtures of the ethanolic leaf extracts of Camellia sinensis and Vicia faba[10].

Method:
Gas chromatography (GC) analysis was carried out using Perkin Elmer Clarus SQ8C gas chromatography equipped with CAPILLARY PTV Injector. The chromatograph was fitted with DB 5 MS capillary standard nonpolar column (30 m x 0.25 mm i.d. x 0.25 µm film thickness). The injector temperature was set at 250 ºC and the oven temperature was initially set at 70 ºC then programmed as follows
Helium was used as a carrier gas with the flow rate of 1 mL/min. One microlitre of the sample (diluted with 1:4) was injected in the SPLIT mode in the ratio of 1:12. The mass spectrometer was operated in the electron impact mode at +VE. Ion source and transfer line temperature were kept at 220 & 250 °C. The mass spectra were obtained by centroid scan of the mass range from 40 to 650 amu. The extract was identified based on the comparison of Retention time (RT) & their obtained mass spectra to NIST library data of the GC-MS system and literature data.

V Results and Discussion:
5.1 Collection of plant
Healthy fresh leaves of *Camellia sinensis* and *Vicia faba* were collected from Nilgiri District. The plant leaves were cleaned, air-dried at room temperature for two weeks, coarsely powdered, transferred to air tight bags and keep in a cool place.

5.2 Extraction of plant material
5g of powdered leaf of *Camellia sinensis* and *Vicia faba* was extracted with 50ml of ethanol.

5.3 QUALITATIVE PHYTOCHEMICAL ANALYSIS

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Ethanol leaf extracts of</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Camellia sinensis</em></td>
</tr>
<tr>
<td>Alkaloids</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Glycosides</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
</tr>
<tr>
<td>Aminoacids</td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
</tbody>
</table>

Table 2: Phytochemical screening of leaf extracts of *Camellia sinensis* and *Vicia faba*

Each leaf extracts of *Camellia sinensis* and *Vicia faba* were analysed for the following phytochemicals:
Table 2 shows the preliminary phytochemical analysis of *Camellia sinensis* and *Vicia faba* using different solvents. Ethanolic extraction gives the best result when compared to methanol and aqueous. Thus the ethanolic extraction is used for the further analysis.
5.4 QUANTITATIVE ANALYSIS

GC-MS:

**Figure 3: Graph of GC-MS analysis on ethanolic extracts of Camellia sinensis**

<table>
<thead>
<tr>
<th>S.No</th>
<th>RETENTION TIME</th>
<th>PEAK AREA (%)</th>
<th>NAME OF THE COMPOUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.013</td>
<td>0.544</td>
<td>Desulphosinigrin</td>
</tr>
<tr>
<td>2</td>
<td>12.842</td>
<td>1.213</td>
<td>Dodecanoic acid</td>
</tr>
<tr>
<td>3</td>
<td>18.660</td>
<td>10.431</td>
<td>Caffeine</td>
</tr>
<tr>
<td>4</td>
<td>21.171</td>
<td>13.733</td>
<td>n-Hexadecanoic acid</td>
</tr>
<tr>
<td>5</td>
<td>24.437</td>
<td>8.471</td>
<td>Oleic Acid</td>
</tr>
<tr>
<td>6</td>
<td>24.897</td>
<td>11.244</td>
<td>Octadecanoic acid</td>
</tr>
<tr>
<td>7</td>
<td>31.150</td>
<td>2.203</td>
<td>9-Octadecenoic acid (Z)-, 2,3-bis(acetyloxy)propyl ester</td>
</tr>
<tr>
<td>8</td>
<td>32.845</td>
<td>2.043</td>
<td>7,8-Epoxylanostan-11-ol, 3-acetoxy-</td>
</tr>
<tr>
<td>9</td>
<td>33.590</td>
<td>5.727</td>
<td>Ethyl iso-allocholate</td>
</tr>
<tr>
<td>10</td>
<td>34.191</td>
<td>7.043</td>
<td>Dodecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester</td>
</tr>
</tbody>
</table>

Table 3: Compounds identified in ethanolic extract of Camellia sinensis by GCMS analysis

**Figure 4: Graph of GC-MS analysis on ethanolic extracts of Vicia faba**

<table>
<thead>
<tr>
<th>S.No</th>
<th>RETENTION TIME</th>
<th>PEAK AREA (%)</th>
<th>NAME OF THE COMPOUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12.832</td>
<td>1.063</td>
<td>Dodecanoic acid</td>
</tr>
<tr>
<td>2</td>
<td>18.650</td>
<td>2.599</td>
<td>Caffeine</td>
</tr>
<tr>
<td>3</td>
<td>21.151</td>
<td>16.132</td>
<td>n-Hexadecanoic acid</td>
</tr>
<tr>
<td>4</td>
<td>23.917</td>
<td>0.719</td>
<td>3,7,11,15-Tetramethyl-2-hexadecen-1-ol</td>
</tr>
<tr>
<td>5</td>
<td>24.432</td>
<td>6.425</td>
<td>9-Octadecenoic acid, (E)-</td>
</tr>
<tr>
<td>6</td>
<td>24.537</td>
<td>3.194</td>
<td>trans-13-Octadecenoic acid</td>
</tr>
<tr>
<td>7</td>
<td>24.892</td>
<td>14.476</td>
<td>Octadecanoic acid</td>
</tr>
<tr>
<td>8</td>
<td>30.970</td>
<td>5.454</td>
<td>a-Sitosterol</td>
</tr>
<tr>
<td>9</td>
<td>31.175</td>
<td>1.792</td>
<td>Ethanol, 2-(9-octadecenlyoxy)-, (Z)-</td>
</tr>
<tr>
<td>10</td>
<td>33.561</td>
<td>6.455</td>
<td>Campesterol</td>
</tr>
</tbody>
</table>

Table 4: Compounds identified in ethanolic extract of Vicia faba by GCMS analysis

Table 3 and 4 shows the results of GC-MS for the ethanolic extracts of Camellia sinensis and Vicia faba. Major active compounds are listed based on the retention time and peak area percentage.
VI Summary and Conclusion:

*Camellia sinensis* and *Vicia faba* are evergreen plants considered as a potent source of plants with medicinal properties. Qualitative analysis of *Camellia sinensis* and *Vicia faba* ethanolic leaf extracts showed the presence of various phytochemicals. GC-MS analysis of ethanolic leaf extracts shows the presence of many major compounds.

VII Bibliography:


