GC-MS Analysis of Microwave Assisted Ethanolic Extract of Pithecellobium dulce

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ABSTRACT

Ten compounds, (1) 2, 5, 6-trimethyl 1, 3-oxathiane, (2) trans-3-methyl-2-N-propylthiophane, (3) 2-furan carboxaldehyde-5-(hydroxymethyl), (4) D-pinitol, (5) heptacosanoic acid, (6) hexadecanoic acid, (7) tetracosanol, (8) 22-tricosenoic acid, (9) methyl-2-hydroxy icosanoate and (10) stigmasterol were identified from the ethanolic extract from the fruits of Pithecellobium dulce. The presence of these compounds was confirmed with the NIST library values. The phytochemical analysis confirms the presence of phenolic, flavonoid and carbohydrates. The present study reveals the use of microwave extraction method for the determination of phenols present in the Pithecellobium dulce. The bio activity of these chemical constituents from the literature reveals that the plant would be a potential source for medicine in the future. This is further confirmed by antioxidant potential of the crude extract which was performed in the present study.

Keywords: Microwave assisted extraction, antioxidant activity, GCMS analysis, Pithecellobium dulce

1. INTRODUCTION

Medicinal plant plays an important role in the development of new drug. Natural product could be potential drug for human and livestock and their analogues can acts as an intermediate for the synthesis of useful drugs. Plant possess many phyto constituent with various biological activity including antioxidant, anti diabetic, anticancer etc., one among such medicinal plant is Pithecellobium dulce, which belongs to the family fabaceae, is an evergreen spiny tree, which grows upto the height of 18-20m and it is native to Mexico, America and also cultivated in the plains of India. It is known as manila tamarind or madras thorn by the locatives of India. The parts of the plant possess various medicinal properties like antioxidant, antiulcerogenic, anti diabetic, anti-venom and estrogenic activities [1-13]. The fruits of Pithecellobium dulce are rich with nutritional and medicinal value. The fruits are consumed as a food in many parts of India, because of its sweet taste and medicinal property [4]. The present work has been undertaken to evaluate the phyto constituents present in the fruits using Gas chromatography and mass spectrometer and to evaluate the in-vitro antioxidant activity of the alcohol extract.

2. MATERIALS AND METHODS

2.1. Collection of plant material

The fruits of Pithecellobium dulce were collected from the local flora in Vellore district, Tamil Nadu in...
the month of March. The sample was dried at room temperature under shade and powdered using mechanical blender and sieved to get uniform particle size.

2.2. Chemicals

Gallic acid, quercetin, ethanol, folin-ciocalteu reagent, sodium carbonate, aluminium chloride, potassium acetate, ascorbic acid, sulphuric acid, ammonium molybdate, sodium phosphate. All the chemicals including solvents were of analytical grade.

2.3. Microwave assisted extraction

About 20gms of dried powdered fruit material was taken and extracted with 200ml of 80% ethanol by microwave assisted soxhlet extraction (Catalyst system CATA R). Microwave oven parameters that were used are: temperature: 50% (350 Watts), Time: 3h. These parameters were optimised in our earlier studies. The extract was concentrated on rotary evaporator under reduced pressure and evaporated to dryness. The dried residue was stored in the refrigerator at 4˚C for further use.

2.4. Preliminary phytochemical screening

Crude extract of *P. dulce* was dissolved in ethanol and subjected to the phytochemical tests. The study was carried out by using standard procedures described by kokate (1986), Harborne (1999) and Evans (1999).

2.5. Determination of total phenolic content

The total phenolic content of *Pithecellobium dulce* fruit extract was determined by using folin-ciocalteu reagent. Gallic acid is used as a reference standard for plotting calibration curve. The total phenolic content was expressed as mg/g gallic acid equivalent of dry extract [13].

2.6. Determination of total flavonoid content

The total flavonoid content of *Pithecellobium dulce* fruit extract was determined using colorimetric aluminium chloride method. Quercetin is used as a reference standard for plotting calibration curve. It is expressed as mg/g quercetin equivalent of dry extract [14].

2.7. Total carbohydrate estimation

The total carbohydrate assay of *Pithecellobium dulce* fruit extract was determined by colorimetric phenol sulphuric acid method (Dubois et al., 1956) with slight modification. Dextrose is used as a reference standard. It is expressed as mg/g dextrose equivalent of dry extract [16].

2.8. Total Antioxidant assay

The total antioxidant assay of *Pithecellobium dulce* fruit extract was determined using ammonium molybdate reduction method. Ascorbic acid is used as a reference standard. The antioxidant activity was expressed as the number of equivalents of ascorbic acid [13].

2.9. Chromatographic conditions

GC-MS analysis was carried out on a Perkin elmer clarus 680 GC-MS instrument employing the following condition: column elite-5MS (30.0m, 0.25 mm ID, 250 μm, operating in electron impact mode; helium was used as a carrier gas at a constant flow and split ratio is 10:1 ; injector temperature is 250°C; flow rate is 1 ml/min; oven temperature is initially 60˚C for 2 min, ramp 10˚C/min to 300˚C, hold for 6 min. Total run time is 32.00 min The molecular weight and structure of the compounds were ascertained by interpretation using the database of National Institute Standard and Technology (NIST).

3. RESULTS AND DISCUSSION

The microwave assisted extraction followed in this work reveals the presence of ten low molecular weight compounds except stigmasterol (m/z-412). This is the first time that the presence of these compounds is identified from this plant *Pithecellobium dulce*. Phytochemical screening of ethanol extract from *Pithecellobium dulce* by qualitative studies shows the presence of phenols, flavonoids, saponins and carbohydrates. The presence of other constituent is not detected in this extract.

3.1. Total phenolic content

Total phenolic content was estimated using folin-ciocalteu reagent. Ethanol extract of pithecellobium dulce pods contain 622.5 mg/g GAE. Among the various phyto constituents that were quantitatively analysed phenolic content is found to be more than the other constituents, this supports our phytochemical analysis indicating the presence of quantifiable amount of phenols in the alcoholic extract (Figure 1).

3.2. Total flavonoid content

Total flavonoid content was estimated using colorimetric aluminium chloride method. Ethanol extract of *Pithecellobium dulce* contains 2.89 mg/g (Figure 1).
3.3. Total carbohydrate content

Total carbohydrate content was estimated by phenol sulphuric acid method. Ethanolic extract of *P. dulce* contains 34.83 mg/g (Figure 1).

3.4. Antioxidant activity

The antioxidant activity was estimated by ammonium molybdate assay. IC$_{50}$ % of inhibitory concentration of ethanol extract is found to be 167.05 mg/g. The antioxidant activity of the extract might be due to the rich phenolic content of the extract (Figure 1).

![Figure 1: Total phenolics, flavonoids, carbohydrates and antioxidant activity of *Pithecellobium dulce* alcoholic extract.](image1)

3.5. GC-MS analysis

The GC-MS chromatogram of ethanolic *P. dulce* extract gave ten peaks which are shown in fig 2. The active principle with their retention time, molecular weight and structure are shown in table 2.

![Figure 2: GC-MS chromatogram for the crude ethanolic extract of *Pithecellobium dulce*.](image2)

Ten compounds were detected in the ethanolic extract of *Pithecellobium dulce*. The GC spectral study revealed the presence of ten compounds include; (1) 2, 5, 6-trimethyl 1, 3-oxathiane, (2) trans-3-methyl-2-N-propulthiophane, (3) 2-furan carboxaldehyde 5 (hydroxymethyl), (4) pinitol, (5) heptacosanoic acid, (6) hexadecanoic acid, (7) tetracosanol, (8) 22-tricosenoic acid (9) methyl-2-hydroxy icosanoate, (10) stigmasterol. Among the identified phyto constituent 2-furan carboxaldehyde 5-(hydroxymethyl) and hexadecanoic acid are reported to possess antibacterial activity, heptacosanoic acid possess antifungal activity, Pinitol possess anti-diabetic activity, tetracosanol possess anti-inflammatory activity and stigmasterol posses’ antineoplastic activity.

4. CONCLUSION

The extraction method present here is simple, rapid and inexpensive, which reduce solvent consumption. Hence proves the green chemistry. In the present study ten compounds were identified from the ethanol extract of the fruits of *Pithecellobium dulce* by gas chromatogram and mass spectrometry (GC-MS) analysis. Thus the microwave assisted extraction method adopted in this present paper demonstrates the detection of volatile and low molecular mass phyto chemicals. The reason might be due to the quick method of extraction with lesser time that has been traditionally followed. This could be possibility of reporting these compounds for the first time by us. The study further confirms the presence of phenols, carbohydrates and flavonoids with remarkable antioxidant activities. The amount present is found to be in the order of Phenols 622.5 mg/g > carbohydrates 34.83 mg/g > flavonoids 2.89 mg/g. Hence, isolation of these compounds would be a way for discovery of new drug molecules.

Acknowledgement

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Conflict of Interest

The authors declare that they have no conflicts of interest.
Table 1. Phytochemical screening of *Pithecellobium dulce* fruit extract

<table>
<thead>
<tr>
<th>S.No</th>
<th>Test</th>
<th>Ethanol extract</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Phenols</td>
<td>+++</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrates</td>
<td>++</td>
</tr>
<tr>
<td>5</td>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Triterpenoids</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Fats and oils</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Saponins</td>
<td>++</td>
</tr>
</tbody>
</table>

Table 2. Identified phytoconstituent from the crude ethanolic extract of *Pithecellobium dulce*

<table>
<thead>
<tr>
<th>S.No</th>
<th>RT value</th>
<th>Name of the compound</th>
<th>Structure of the compound</th>
<th>Molecular weight</th>
<th>References</th>
<th>Therapeutic activity/chemical property</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.5</td>
<td>2,5,6-trimethyl-1,3-oxathiane</td>
<td><img src="image" alt="Structure" /></td>
<td>146</td>
<td>Zhen chem et al., US 20120183490 A1</td>
<td>Aroma</td>
</tr>
<tr>
<td>2</td>
<td>8.1</td>
<td>trans-3-methyl-2-N-propylthiophane</td>
<td><img src="image" alt="Structure" /></td>
<td>144</td>
<td>Guor jien wei et al., 2011</td>
<td>Aroma</td>
</tr>
<tr>
<td>3</td>
<td>9.5</td>
<td>2-furan carboxaldehyde 5(hydroxymethyl)</td>
<td><img src="image" alt="Structure" /></td>
<td>126</td>
<td>Moussa ahmed et al., 2013</td>
<td>Anti-bacterial</td>
</tr>
</tbody>
</table>
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4 12.9 D-Pinitol 194 Selvaraj sivakumar et al., 2009 Antidiabetic

5 15.2 Heptacosanoic acid 410 Maya kushawaha et al., 2012 Anti-fungal

6 17.8 Hexadecanoic acid 256 Zhong-hui pu et al., 2010 Anti-bacterial

7 23.9 22-Tricosenoic acid 352 - -

8 25.3 Tetracosanol 354 Montserat-de et al., 2014 Anti-inflammatory

9 26.7 Methyl-2-hydroxyicosanoate 341 - -

10 28.6 Stigmasterol 412 Tereza cristina da silva et al., 2005 Anti-pyretic, Anti-neoplastic

**References**


4. Shankar D Katekahaye, Maheshkumar S Kale (2012). Antioxidant and free radical...


