Phytochemical and Metabolite Fingerprinting of *Helictres isora*
Through Gas chromatography and Mass Spectrum analysis

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ABSTRACT

Objectives: In the present study, phytochemical fingerprinting of methanolic extracts from *H. isora* fruits were investigated through Gaschromatography and mass spectrometric (GC-MS) studies. Methods: Methanolic extracts of *H. isora* were subjected for GC-MS fingerprinting to screen out the metabolite present in the extract. Results: GC-MS results revealed that the extract consists of alkaloid and phenolic derivatives as its key metabolite. All above antidiabetic agent of berberine chloride derivative of dimethylene berberine is reported in the present investigation. Conclusion: *H. isora* seems to possess significant phytochemicals which have key importance in pharmacological industry for treating several disease like diabetes, cancer and aging related ailments.

KEY WORDS: *H. Isora*, antidiabetes, antioxidant, GC-MS, phytochemicals.

1. INTRODUCTION

In Southeast Asia, despite the increasing availability of modern medicine, the use of traditional medicine remains popular as access to modern medicine is widespread but not available to all.¹ People with low socioeconomic status are generally believed to rely more on traditional medicine because of inaccessibility and unavailability of health care services. In fact, all member countries of the Southeast Asian region, with assistance from WHO, are developing, strengthening and introducing the use of traditional medicine into primary health care as these are inexpensive and readily available compared to their pharmaceutical alternatives.² Herbal medicines, which include herbs, herbal preparations and herbal products, are the most widespread of traditional medicines and women their most frequent users.³ Herbal medicines are used by women to treat a number of reproductive health problems, such as menstrual problems, infertility, discomforts and dysfunctions of pregnancy, labor and menopause.⁴

*Helictres isora* L. is a large arborescent shrub which is being used as an antigastropasmodic, antihelmintic, antispasmodic, antipyretic, anti diarrhoeal, antisyneretic and as a tonic after childbirth.⁵ Stems of this plant are used as anthemthic, colic, and aphanth, while fruits are used as colic, anticonvulsant, and abdominalgia.⁶ Traditionally, the root juice is claimed to be useful in diabetes, emphysema, and snake-bite.⁷ From the roots, betulinic acid, daucosterol, sitosterol, isorin were isolated.⁸ Cucurbitacin B and isocucurbitacin B were isolated and reported to possess cytotoxic activity.⁹ In addition, Hattori and co-workers reported an inhibitory activity of the water extract of fruits of *H. isora* against reverse transcriptase from avian myeloblastosis virus and antiHIV-1 activity.¹⁰ Six neolignans, the helicterins A-F were isolated from aqueous extract of the fruits,¹¹ plant also contains flavonoid and glucosides.¹² Aqueous extract of *H. isora* improves the level of plasma insulin, decrease glucose levels, reverses the changes in the levels of the carbohydrate moieties of glycoproteins and protein marker enzymes, and possess glycaemic control and renoprotective activity in streptozotocin-induced diabetic rats.¹³ The present study was undertaken to quantify the phytochemical constituents of methanolic extract of *H. isora* through Gas chromatography and Mass spectrometric fingerprinting.

2. MATERIALS AND METHODS

Chemicals: All the chemicals used in this experiment are of HPLC grade chemical of analytical grade. Methanol (MeOH, HPLC) were purchased from Fischer. Milli-Q H₂O was purchased from Millipore.

2.1. Plant collection and extraction:
*H. isora* fruits were collected from agricultural fields around Chidambaram was made to shade dried. Shade dried materials were pulverized to fine powder with blender. Powdered fruit material were extracted with methanol (w: v) following the methodology of Na with slight modification. The resultant residues were then concentrated using solvent evaporator. The extract of 1 mg/ml of extract was prepared by dilution of the stock with sterile DMSO (Dimethyl sulfoxide) and utilized for pharmacological assays.
2.2. Gas chromatography and Mass spectrometric fingerprinting

GC-MS sample was prepared by dissolving about 1 mg of *H.isora* extract in 5 mL of methanol. Active extract was dissolved in HPLC grade methanol and subjected to GC and MS JEOL GC mate equipped with secondary electron multiplier. JEOL GCMATE II GC-MS (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was used with fused silica 50 m x 0.25 mm I.D. Analysis conditions were 20 minutes at 100°C, 3 minutes at 235°C for column temperature, 240°C for injector temperature, helium was the carrier gas and split ratio was 5:4. The sample (1µl) was evaporated in a split less injector at 300°C. Run time was 22 minutes. The components were identified by gas chromatography coupled with mass spectrometry. Interpretation of mass spectra of GC-MS was done using the database of National Institute Standard and Technology (NIST) library search which is having more than 62,000 drug formulation. The mass spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST-08 and Wiley-08 libraries. The name, molecular weight and structure of the components of the test materials were validated.

3. RESULTS

3.1. GC-MS based metabolite fingerprinting

Gas chromatographic fingerprinting of methanolic extract of *H.isora* revealed that extract seems to contain alkaloid and terpenoid derivatives most of which consist of phenolic and amide derivatives (Table.1). *H.isora* consists of 38 different metabolites of which Methyl 4-methyl-2-(2'-nitrosophenyl)-5-oxo-5,7-dihydrofuro[3,4-b]pyridine-3-carboxylate (0.44%), 3-(D-Galacto-penta-O-acetylpentitol-1’-yl)-4-nitropyrrle (0.63%), 3-(D-Manno-penta-O-acetylpentitol-1’-yl)-4-nitropyrrle (1.43%), Phenylcyclopentadienyl (2.9%), Diphenyl r-2-methoxycarbonyl-2,1,5-diphenylpyrrolidine-c-3,t-4-dicarboxylate (3.6%), Docosanoic acid, 1,2,3-propanetriyl ester (6.2%), 2-Propenoic acid, 2-ethylhexyl ester (4.11%), Dimethylene-berberine (1.29%) etc., These derivatives are rich in –OH groups which are highly reactive with deleterious free radicals which in turn sequester these free radicals from the physiological systems.

<table>
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<th>S.No</th>
<th>Metabolite</th>
<th>Area</th>
<th>Structure</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Methyl 4-methyl-2-(2’-nitrosophenyl)-5-oxo-5,7-dihydrofuro[3,4-b]pyridine-3-carboxylate</td>
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<tr>
<td>2</td>
<td>3-(D-Galacto-penta-O-acetylpentitol-1’-yl)-4-nitropyrrle</td>
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<tr>
<td>4</td>
<td>Phenylcyclopentadienyl</td>
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<tr>
<td>5</td>
<td>Diphenyl r-2-methoxycarbonyl-2,6 diphenylpyrrolidine-</td>
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<tr>
<td></td>
<td>c-3,t-4-dicarboxylate</td>
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</tr>
<tr>
<td>6</td>
<td>Docosanoic acid, 1,2,3-propanetriyl ester</td>
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<tr>
<td>7</td>
<td>2-Propenoic acid, 2-ethylhexyl ester</td>
<td>4.11</td>
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<tr>
<td>8</td>
<td>Dimethylene-berberine</td>
<td>1.29</td>
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4. DISCUSSION

Plants are rich in variety of phytochemicals such as phenolics and flavonoids, which provide health benefits. Significantly, many studies suggest that these compounds are important antioxidant substances that act as reducing agents, singlet oxygen quenchers or electron donors with chelating properties. Plants polyphenols are secondary metabolism products and they constitute one of the most numerous and widely distributed groups of natural antioxidants. Polyphenols act as reducing agents and antioxidants in vitro via several mechanisms including the scavenging of free radicals, chelation of transition metals, as well as the mediation and inhibition of enzymes.

The use of traditional medicinal plants present a large source of natural antioxidants that might serve as leads for the development of novel drugs. The root juice of *H. isora* has been used in Indian folkloric medicine for the treatment of diabetes, but no previous pharmacological studies have been carried out to elucidate its mode of action. The present study reveals the presence of dimethylene berberine a berberine chloride derivative which is a potent antidiabetic agent. The radical scavenging activity of *H. isora* and its anticancer activity seem to be correlated with its polyphenolic constituents though its active components could play important roles in its antioxidative effect. Consequently, it is possible that the total phenolic constituents may contribute to antioxidant and anti-cancer activity of course. In previous studies, the presence of phenolic substances including flavonoids and tannins were reported in *H. isora*. An In-vitro system of antioxidant effect of *H. isora* in different free radical scavenging system exhibited maximum radical quenching activity.

5. CONCLUSION

From the present study, it is evident that the methanol extract of *H. isora*, fruits consists of bioactive metabolites which is evident based from its GC-MS based metabolite identification. This finding substantiates its traditional uses in treating various disorders and increases the interest and potential use of this sample as nutraceutical and pharmacological agent. Further isolation and purification of compounds from this extract and study of their biological effects may provide further information of their medicinal value.

6. Conflicts of interest

All authors have none to declare.

7. ACKNOWLEDGMENTS

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8. REFERENCES


Source of support: Nil, Conflict of interest: None Declared