ISOLATION, CHARACTERIZATION OF PHYTOCONSTITUENTS AND PHARMACOCOLOGICAL SCREENING OF IPOMOEA STAPHYLINA

DURGA PAVANI REDDY1,2, RAMAKRISHNA KOTA1*, RENUKA S3, SNEHA JIJABAPU ANARTHÉ2, NULGUMNALLI MANJUNATHAIAH RAGHAVENDRA1

1Department of Pharmaceutical Chemistry, 2Pharmacognosy Gokaraju Rangaraju College of Pharmacy, Osmania University, Bachupally, Kukatpally, Hyderabad-500090, Andhra Pradesh, India, 3Laila IMPEX, Vijayawada, Email: krk.rama@gmail.com

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ABSTRACT

The present study evaluates the methanolic and hydro alcoholic extracts of whole plant, stem, root, flower, leaves of Ipomoea staphylina for their in vitro antioxidant activity (by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) and superoxide radical scavenging method), in vitro anti diabetic activity (by α-glucoisidase and α-amylase inhibitory method) and in vitro anti inflammatory activity (by 5-lipoxygenase inhibitory method). DPPH radical scavenging activity of stem hydro alcoholic and leaf methanolic extract of Ipomoea staphylina is found to be more potent as compared to other extracts with IC50 values 17.67 µg/ml, 32.77 µg/ml respectively. Superoxide radical scavenging activity of stem hydro alcoholic and leaf hydro alcoholic extracts of Ipomoea staphylina is also found to be potent compared to other extracts with IC50 values 15.54 µg/ml, 16.02 µg/ml respectively. So methanolic extract of Ipomoea staphylina leaves was subjected to column chromatography and two compounds namely Sitosterol-3-O-β-D-glucoside (1) and chiro deoxy inositol (2) were isolated and characterized by various spectroscopic and chromatographic techniques. These two constituents are first reported in Ipomoea staphylina.

Keywords: Ipomoea staphylina, Antioxidant activity, Anti-inflammatory activity, Anti-diabetic activity.

INTRODUCTION

Diabetes is a metabolic disorder associated with an increased risk of developing vascular and other health complications. Oxidative stress and inflammation are the major pathogenetic mechanisms considered to be implicated in diabetes1,2. Subjects with type 2 diabetes have been shown to have increased levels of lipid peroxidation, oxidative damage to DNA and protein oxidation3, presumably caused by an over production of free radicals and a decreased antioxidative defence. Enhanced production of free radicals is related to hyperglycaemia, insulin resistance and hyperinsulinemia4. High levels of glucose lead to an increased production of free radicals via different mechanisms such as glucose autooxidation and formation of advanced glycation end products5.

Besides oxidative stress, inflammation is also implicated in the development of complications in type 2 diabetes. Cyclo-oxygenase catalyzed PG formation, and subsequently low-grade inflammation is suggested to be an early event in the development of type-2 diabetes which is further linked to oxidative stress6. Elevated levels of high sensitivity C-reactive protein (hsCRP) and IL-6 are seen in subjects with type-2 diabetes6, and also associated with an increased risk for developing the other diseases in future7. However inflammation and oxidative stress are related to glycaemic control is still not fully clarified.

Ipomoea staphylina is commonly found on hedges and bushes in the forests and waste lands. It is a perennial, woody and glabrous shrub with pink flowers. Traditionally Ipomoea staphylina is used for respiratory disorders8,9. Traditionally genus Ipomoea is used as purgative, dyspepsia, antihelmintic, bronchitis10.

So in present study Ipomoea staphylina is evaluated for in vitro antioxidant, antidiabetic and anti-inflammatory pharmacological activities.

MATERIALS AND METHODS

**Chemicals and instruments** - 1H and 13C-NMR, H-H correlation spectroscopy (COSY), NOE and HMBC spectra were recorded on an Bruker Avance 400 MHz spectrometer in D2O, unless otherwise stated. Chemical shifts are shown in δ values (ppm) with tetramethylsilane (TMS) as an internal reference. Mass spectra were recorded on AgIlant 1100 Series LC-MS spectrometer. IR spectra were recorded on a Perkin Elmer Spectrum BX, FT-IR System. TLC analyses were performed on Kieselgel 60 F254 (Merck) plates (silica gel: 0.25 mm layer thickness); compounds were visualized by dipping plates into 20% (v/v) H2SO4 reagent and then heated at 110°C for 5-10 min. Silica gel (Merck 60A; 60-120 mesh) was used for column chromatography. All chemicals were procured from Merck India Pvt. Ltd.

**Plant Material**

Whole plant, stem, root, flower and leaves of Ipomoea staphylina were collected from Malavaram, Krishna district, AP, India in January 2010. Voucher specimens were deposited at the herbarium of the Nutraceuticals, Laila Impex, R&D centre, Vijayawada, AP, India. The collected plant material was authenticated by Dr. K. K. Reddy, Scientist, Dept of Taxonomy, Laila impex, R&D centre. The plant was dried under shade at Dept. of Taxonomy, Laila impex, R&D centre.

**Extraction, Fractionation and Isolation**

Different parts of Ipomoea staphylina plant (500g each) were refluxed with methanol and hydro alcohol (60%/methanol in water); and different extracts so obtained were named as ISWM (whole plant methanolic extract), ISWH (whole plant hydroalcoholic extract), ISSM (stem methanolic extract), ISSH (stem hydro alcoholic extract), ISRM (root methanolic extract), ISRH (root hydro alcoholic extract), ISLM (leaves methanolic extract), ISLH (leaves hydro alcoholic extract), ISFM (flower methanolic extract), ISFH (flower hydro alcoholic extract). Then all the extracts were subjected to in vitro antioxidant, anti diabetic and anti-inflammatory pharmacological activities. Based on the results of invitro activities leaves of the plant Ipomoea staphylina is selected for isolation of compounds by column chromatography.

The dried leaves of Ipomoea staphylina (500 g) were refluxed in methanol (MEOH) for 4 h (4 x ) The total filtrate was then concentrated to dryness in vacuo at 40°C to render the MEOH extract (40 g). A portion of methanolic extract (35 g) was first chromatographed over a Silica gel column, using 80% ethyl acetate in hexane, ethyl acetate, 1% methanol in ethyl acetate, 5% methanol in ethyl acetate, 8% methanol in ethyl acetate and 10% methanol in ethyl acetate to afford eleven fractions (F01-F11). The ethyl acetate fraction (F3) gave Sitosterol-3-O-β-D-glucoside 1 (30 mg). The 8% methanol in ethyl acetate fraction (F9) gave deoxy chiro inositol 2 (20 mg). The isolated compounds were identified and characterized by spectroscopic methods including IR, NMR and MASS; Compounds Sitosterol-3-O-β-D-glucoside 1 (1) and Deoxy chiro inositol 2 (2) were identified by comparing the data with published data (Fig. 1).
In vitro studies

Anti-diabetic Activity

α-Glucosidase inhibitory activity

The enzyme α-glucosidase inhibitory activity is determined by incubating solution (0.1 mL) of an enzyme preparation with 0.2 M Tris buffer, pH 8.0 (1.0 mL) containing various concentrations of extract at 37 °C for 60 minutes by using glucose as working standard. The reaction mixture is heated for two minutes in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidation method15. (Assay condition 37 °C ± 0.1 °C, pH 8.0, O.D at 540 nm). The percentage inhibition is calculated as:

\[
\% \text{ inhibition} = \frac{\text{Enzyme activity of the control} - \text{Enzyme activity of the extract}}{\text{Enzyme activity of the control}} \times 100
\]

α-Amylase inhibitory activity

A starch solution (0.1% w/v) was obtained by stirring 0.1 g of potato starch in 100 mL of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of α-amylase in 100 mL of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartrate solution and 3.5 mL nitro salicylic acid solution 96 mM. Both control (Acarbose) and plant extracts were added with starch solution and left to react with α-amylase solution under alkaline conditions at 25 °C. The reaction was measured over 3 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3-amino-5- nitro salicylic acid. This reaction is detectable at 540 nm16. (Temperature 25 °C ± 0.1 °C, pH 4.8; O.D at 540 nm).

\[
\% \text{ inhibition} = \frac{\text{Enzyme activity of the control} - \text{Enzyme activity of the extract}}{\text{Enzyme activity of the control}} \times 100
\]

Anti-oxidant Activity

DPPH Radical Scavenging Activity

DPPH radical scavenging activities of all the fractions were determined by the method of Blois14. Initially, 0.2 mL of the fractions at a concentration of 25, 50, 75 and 100 µg/mL was mixed with 1 mL of 0.2 mM DPPH (dissolved in methanol). The reaction mixture was incubated for 20 minutes at 28 °C under dark condition. The control contained all reagents except the extract fraction while methanol was used as blank. The DPPH radical scavenging activity was determined by measuring the absorbance at 517 nm using a spectrophotometer. The DPPH radical scavenging activity (%) of the sample was calculated as:

\[
\% \text{ DPPH scavenging activity} = 1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}} \times 100
\]

Superoxide Anion Radical Scavenging Activity

Superoxide radical scavenging activity of leaf methanolic extract of Ipomoea staphylina was determined by Nitro Blue Tetracromol (NBT) riboflavin photo reduction method of Mccord and Fridovich17. The assay mixture contained EDTA solution (6.6 mM) containing NaCN (3 µg), riboflavin (2 µM), NBT (50 µM), test substances and phosphate buffer (67 mM, pH 7.8) in a final volume of 3 mL. The absorbance at 560 nm were measured before and 15 min after illumination (Table 1). All tests were run in triplicate and mean values were used to calculate percentage scavenging ability and IC50 values were calculated using linear regression analysis.

The superoxide anion radical scavenging activity (%) was calculated as:

\[
\% \text{ superoxide scavenging activity} = 1 - \frac{\text{absorbance of sample}}{\text{absorbance of control}} \times 100
\]

Table 1: IC50 values of methanolic and hydro alcoholic extracts of Ipomoea staphylina stem (ISSM, ISSH), flower (ISFM, ISFH), leaves (ISLM, ISRH), root (ISRM, ISRH), whole plant (ISWM, ISWH) for various in vitro antioxidant, antidiabetic and anti-inflammatory activities.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Sample</th>
<th>DPPH IC50 (µg/ml)</th>
<th>Superoxide IC50 (µg/ml)</th>
<th>α-glucosidase IC50 (µg/ml)</th>
<th>α- amylase IC50 (µg/ml)</th>
<th>5-Lox IC50 (µg/ml)</th>
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<tr>
<td>1</td>
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<td>17.67</td>
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<td>46.76</td>
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<td>&gt;25</td>
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<td>2</td>
<td>ISSM</td>
<td>37.87</td>
<td>29.06</td>
<td>74.78</td>
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<td>&gt;25</td>
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<td>3</td>
<td>ISFM</td>
<td>63.85</td>
<td>89.64</td>
<td>73.46</td>
<td>148.7</td>
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<td>ISFH</td>
<td>81.03</td>
<td>31.17</td>
<td>&gt;150</td>
<td>&gt;200</td>
<td>&gt;20</td>
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<td>5</td>
<td>ISLM</td>
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<td>&gt;25</td>
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<td>ISLH</td>
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<td>88.05</td>
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<td>&gt;10</td>
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<td>&gt;10</td>
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<td>97.00</td>
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<td>29.80</td>
<td>91.50</td>
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<td>11</td>
<td>Std</td>
<td>3.41</td>
<td>0.62</td>
<td>0.009</td>
<td>33.59</td>
<td>3.97</td>
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</table>

Anti-inflammatory Activity

In vitro 5-Lipoxygenase inhibition

5-LOX enzyme inhibitory activity of Ipomoea staphylina extracts was measured using the method of Reddanna et al.20 modified by Ulusu et al.21. The assay mixture contained 80 mM linoleic acid and 10 µl potato 5-LOX in 50 mM phosphate buffer (pH 6.3). The reaction was initiated by the addition of enzyme buffer mix to linoleic acid and the enzyme activity was monitored as the increase in absorbance at 234 nm. The reaction was monitored for 120 sec and the inhibitory potential of the test substances was measured by incubating various concentrations of test substances for two minutes before addition of linoleic acid. All assays were performed in triplicate. Percentage inhibition was calculated by comparing slope of test substances with that of enzyme activity.

RESULTS AND DISCUSSION

In the DPPH radical scavenging assay, antioxidants react with the DPPH radicals, which is stable free radical and exists naturally in deep violet color, to turn into a yellow colored α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the radical scavenging potential of the antioxidant. DPPH radical scavenging activity of stem hydro alcoholic and leaf methanolic extract of Ipomoea staphylina is found to be potent as compared to other extracts and results are compared with standard as having IC50 values 17.67 µg/mL, 32.77 µg/mL respectively (Fig 2). It has been found that phenolics, flavonoids and tocopherols reduce the DPPH radicals by donating their hydrogen donating ability.

In this study, the superoxide anion scavenging effects of various extracts were analyzed and the results are given in Fig 3. Among the extracts tested, stem hydro alcoholic and leaf hydro alcoholic extract of Ipomoea staphylina is found to be potent as compared to other extracts having IC50 values 15.54 µg/mL, 16.02 µg/mL respectively. The superoxide anion scavenging activity might be due to the action of polyphenolic compounds.
Compound (1): White crystals; IR (KBr), ν ~ 3440, 3311, 1422, 1278, 1069, 1049 cm⁻¹, δ: 3.885 (3H, s), 3.890 (3H, s), 0.80 (3H, d, J = 7.2 Hz), 1.941 (1H, d, J = 14, 4,4Hz), 1.764 (1H, dd, J = 14, 4,4Hz), 3.664 (1H, dd, J = 9, 6Hz), 3.717 (1H, dd, J = 9, 2, 4,4Hz), 3.883 (1H, t, J = 3.6Hz), 3.974 (1H, q, J = 3.6Hz), δ: 33.36 (C-1), 71.14 (C-2), 74.76 (C-3), 69.06 (C-4), 72.39 (C-5), 68.70 (C-6). LC-MS (positive) m/z: 575.5 [M+H].

Compound (2): White solid; IR (KBr), ν ~ 3440, 3311, 1422, 1278, 1069, 1049 cm⁻¹, δ: 3.885 (3H, s), 3.890 (3H, s), 0.80 (3H, d, J = 7.2 Hz), 1.941 (1H, d, J = 14, 4,4Hz), 1.764 (1H, dd, J = 14, 4,4Hz), 3.664 (1H, dd, J = 9, 6Hz), 3.717 (1H, dd, J = 9, 2, 4,4Hz), 3.883 (1H, t, J = 3.6Hz), 3.974 (1H, q, J = 3.6Hz), δ: 33.36 (C-1), 71.14 (C-2), 74.76 (C-3), 69.06 (C-4), 72.39 (C-5), 68.70 (C-6). LC-MS (positive) m/z: 163 [M+H].

On comparing data generated with that of published literature, it was confirmed that compounds isolated from methanolic extract of leaves of Ipomoea staphylina were Sitosteryl-3-O-β-D-glucoside13 (1) and Deoxy chiro inositol 15-15 (2).

Fig. 2: DPPH radical scavenging activity of methanolic and hydro alcoholic extract of Ipomoea staphylina stem (ISSM, ISSH), leaves (ISLM, ISLH), flower (ISFM, ISFH), root (ISRM, ISRH), whole plant (ISWM, ISW). On comparing data generated with that of published literature, it was confirmed that compounds isolated from methanolic extract of leaves of Ipomoea staphylina were Sitosteryl-3-O-β-D-glucoside13 (1) and Deoxy chiro inositol 15-15 (2).

Fig 3: Superoxide radical scavenging activity of methanolic extract of Ipomoea staphylina stem (ISSM) and hydro alcoholic extract of Ipomoea staphylina stem (ISSH), leaves (ISLH), whole plant (ISWH).

Also the in-vitro α-glucosidase inhibitory activity, in-vitro α-amylase inhibitory activity and 5-LOX enzyme inhibitory activity of various extracts were analyzed. The 50 % inhibitory concentration of various extracts of Ipomoea staphylina was found to be very less potent compared to standard. So extracts of Ipomoea staphylina are devoid of in-vitro antidiabetic and anti-inflammatory activity.

Methanolic extract of Ipomoea staphylina leaves was selected for further studies, because methanolic extract was found to be potent for in-vitro antioxidant activity in DPPH and superoxide radical scavenging activity. It was fractionated by column chromatography. Some of the fractions were further subjected to fractionation. Some of the pure compounds were isolated from leaves methanolic extract. The fractions which were obtained in negligible amounts were excluded from fractionation.

Spectral Analysis of Isolated Compounds

Compound (1): White crystals; IR (KBr), ν ~ 3460, 2920, 2849, 1458, 1366, 1021, 1655, 800 cm⁻¹. LC-MS (positive) m/z: 575.5 [M+H].

Compound (2): White crystals; IR (KBr), ν ~ 3440, 3311, 1422, 1278, 1069, 1049 cm⁻¹. LC-MS (positive) m/z: 163 [M+H].

REFERENCES