ICHNOCARPUS FRUTESCENS (LINN) – A PLANT WITH DIFFERENT BIOLOGICAL ACTIVITIES

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ABSTRACT

Context: Ichnocarpus frutescens (Linn) (Family-Apocynaceae) is an evergreen plant and this plant is used in traditional Indian medicine for centuries to treat several diseases such as fever, dyspepsia, headache. The possible antimutator activity and antioxidiant role of I. frutescens in the mice transplanted with Ehrlich ascites carcinoma (EAC) was reported. Objectives: We have evaluated the antimicrobial activity, in vitro biochemical antioxidant activity (viz. superoxide radical scavenging activity, DPPH radical scavenging assay) and in vitro biochemical antioxidant activity (viz. α-glucosidase inhibitory assay) of the ethyl acetate extract of I. frutescens roots. Results: The root extract of the plant exhibited potential antibacterial activity against, Shigella flexneri 16 (gram negative), Shigella dysenteriae 1 (gram negative), Vibrio cholerae non 0139(L4) (gram negative), Vibrio cholerae non 0139(CSK6669) (gram negative), Streptococcus pneumoniae (gram positive) and Escherichia coli (gram negative). The extract also showed significant inhibitory activity of α-glucosidase enzyme compared to acarbose (positive control) which indirectly indicated anti diabetic activity. The extract had potent superoxide radical scavenging activity compared to butylated hydroxyanisole (BHA). Conclusion: These results indicate that this plant may have phytochemicals of potential antibacterial, antioxidiant and anti diabetic activities.

Keywords: Ichnocarpus frutescens, antibacterial, antidiabetic, antioxidiant, superoxide, α-glucosidase, acarbose, butylated hydroxyanisole.

INTRODUCTION

Ichnocarpus frutescens (Linn) R.Br. (Family-Apocynaceae) is an evergreen plant and this plant is used in traditional Indian medicine for centuries to treat several diseases such as fever, dyspepsia, headache. The plant is known as Dudhi, 'Shyamalata' in Bengali, 'Black creeper' in English and 'Ananta,' 'Sarwa' in Sanskrit. This plant is grown wild in the hilly areas of Tripura. Ichnocarpus frutescens leaf, stem and root were investigated for its physicochemical and phytochemical screening. Various parts of this plant are used as a cure for fever, dyspepsia, skin troubles and headache. Laboratory studies have demonstrated that extracts of the plant inhibit tumors, protect liver cells from damage in streptozotocin overdose, and correct hyperglycemia in diabetic rats. It also has analgesic and anti-inflammatory properties, reduces fever, and lowers fasting glucose and improves glucose tolerance in diabetes.

Earlier works with chloroform and methanol extracts of this plant reported the antimutator activity and antioxidiant role against Ehrlich ascites carcinoma (EAC) in mice, in vitro antioxidiant activity of polyphenolic extract of the leaves against U-937 and K-562 cell lines, antioxidiant activity of ethyl acetate fraction of methanol extract of the flower and isolation of saponins from the aerial part. Other several works also reported on this plant, the anti-diabetic activity of aqueous extract of roots of I. frutescens in streptozotocin-nicotinamide induced type-II diabetes in rats, hepatoprotective and antioxidiant activity on paracetamol-induced hepatotoxicity in rats, screening for the antioxidiant property as literature survey revealed that various parts were known for its flavonoid content hence the various extracts, fractions and isolated flavonoids of the flowers of I. frutescens, the wound healing potential on different experimental models of wounds in rats of the methanol root extract, the anti-inflammatory activity of methanolic extract of I. frutescens (MEIF) by carrageenan, and cotton pellet induced granuloma tests to determine its effects on acute and chronic phase of inflammation models in rats, the methanolic extract of I. frutescens R.Br. root (MEIF) for its anti-pyretic potential on normal body temperature and yeast-induced pyrexia in albino rats, the extract of I. frutescens leaves which were tested against streptozotocin-induced diabetic rats, the hepatoprotective effect of chloroform and methanol extract (CEIF and MEIF) of whole plant of I. frutescens (Linn.) by paracetamol-induced liver damage in rats. It was also published that the hexane, chloroform and aqueous extracts from I. frutescens roots were evaluated for their antimycobacterial activity.

In the current study, we have evaluated different biological activities of ethyl acetate extract of the plant roots. These are antibacterial activity, in vitro biochemical antioxidiant activity (viz. superoxide radical scavenging activity, DPPH radical scavenging activity) and antioxidiant activity (viz. α-glucosidase inhibitory assay).

MATERIALS AND METHODS

Collection of plant species

The plant materials were collected from Jolai Barhi, Kali, South Tripura, in the month of March, 2008. The plant was identified by Prof. B. K. Dutta, Taxonomy Lab, Deptt. of Botany, Tripura University.

Extraction method of I. frutescens

The fresh plant roots were washed thoroughly with water to remove adhering mud or dirt. The dirt roots were cut into pieces and shade dried. Shifting of material at regular interval of time was done in order to avoid growth of fungi. Completely dried pieces were powdered in a grinder and passed through mesh size 40 (sieve) in order to obtain uniform size of powdered material. Air dried powdered roots of I. frutescens (2 kg) were extracted with ethyl acetate by percolation method at room temperature for 1 week. The ethyl acetate extract was concentrated under reduced pressure in vaccuo to get a residue (104 gm). The ethyl acetate (EtOAc) extract is used for the study of biological activity.

Bacterial samples

Eight bacterial species viz. Klebsiella pneumoniae (BCH 271), Shigella flexneri 16, Shigella dysenteriae 1, Vibrio cholerae non 0139(L4), Vibrio cholerae non 0139(CSK6669), Streptococcus pneumoniae, Streplococcus aureus and Escherichia coli, collected from National Institute Cholera and Enteric Disease, Kolkata and AG. Govt. Med. College, Agartala, were used for antibacterial assay.

Antibacterial assay

The antibacterial activity of the EtOAc extract of I. frutescens was tested by the disc diffusion method [Duraiappan et al. 2006] on selected microorganisms with some modifications.

Disc Diffusion Method

The extracts of three concentrations, (100 µg, 500 µg and 1000 µg) were prepared using dimethyl sulphoxide (DMSO) as solvent. The test microorganisms (freshly cultured) were seeded into respective medium (SRL Agar) by spread plate method with 40 µl of each strain. After solidification of the agar, paper discs (5 mm in diameter and 0.4 mm in thickness), were placed in different petridishes.
(100 mm in diameter) containing agar media, then each of the different concentrations of the extract was applied on each disc (5 μl). DMSO was also applied as positive control for each concentration of the extract. After that, the plates were incubated at 37°C for 24 hr. The diameters of inhibition zones were measured to evaluate antibacterial activity. All experiments were carried out in triplicate and the mean of the readings were recorded.

**In vitro antioxidant activity and anti-diabetic activity**

The root extract was tested for its in vitro free radical scavenging property using superoxide anions radicals in NADH/PMS/NBT system and stable DPPH free radicals; similarly antidiabetic activity of the extract was evaluated using α-glucosidase inhibitory assay and all experiments were performed five times and the results averaged.

**Chemicals**

All chemicals used were of analytical grade. Nitroblue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), phena zona m ethosulphate (PMS), p-nitrophenyl α-D-glucopyranoside (p-NPG), α-glucosidase, sodium carbonate (Na2CO3), ascorbic acid and all solvents were obtained from SISCO Research Laboratories Pvt. Ltd, Mumbai, India. 1.1- diphenyl-2-picryl hydrazyl (DPPH) was obtained from SIGMA-ALDRICH,USA. Butylated hydroxy anisole (BHA) and methanol were obtained from MÉRK Chemicals, acarbose drug were obtained from Buyers Pharmaceuticals.

**Superoxide radical scavenging activity**

Superoxide scavenging activity of *I. frutescens* extracts were measured according to the method of Robak *et al* with some modifications 22. All the solutions were prepared in 100 mM phosphate buffer (pH 7.4). 0.5ml of NBT (156 μM), 0.5ml of NADH (468 μM), 1.5ml of extracts (to produce final concentrations of 1-200μg/ml) were mixed. The reaction was started by adding 50 μl of PMS (60μM) and the mixture then incubated at 37°C for 5 min followed by measurement of absorbance at 560 nm using UV-VIS Spectrophotometer 117, Systronics India Ltd. Same procedure was used as negative control using distilled water instead of the extracts. Now the percent of inhibition were measured using following formulae. Butyalted hydroxyanisole (BHA) and methanol were obtained from MÉRK Chemicals, acarbose drug were obtained from Buyers Pharmaceuticals.

**DPPH radical scavenging activity**

DPPH radical scavenging activity was measured according to the method of Cotelle *et al* with some modification 21. In brief 1.5ml reaction mixture containing 100μl of DPPH (100μM in methanol) and 1.4 ml of ethyl acetate fraction of root extract [at various concentration; 0.5-50μg/ml] in distilled water was incubated at 37°C for 30 min and absorbance was recorded at 517 nm using UV-VIS Spectrophotometer 117, Systronics India Ltd. The percentage inhibition of DPPH radicals was calculated by comparing the results of the test with those of the control (not treated with extract) using above formulae.Here, L-ascorbic acid used as a positive control was dissolved in distilled water at the same concentration of each extract 17.

**α-glucosidase inhibitory assay**

One hundred microliters of 3 mM p-nitrophenyl glucopyranoside (pNPG) in 0.2 M sodium phosphate buffer (pH 6.8) was added as a substrate to the mixture of 50 μl of α-glucosidase (0.15 unit/ml) and 50 μl of sample to start the reaction. The reaction was conducted at 37°C for 15 min and stopped by the addition of 750 μl of 0.1 M Na2CO3. α-glucosidase activity was assessed by measuring the release of p-nitrophenol from pNPG at 405 nm 18. Acarbose was used as positive control. All tests were performed independently five times (n=5) and data were expressed as mean ±SD.

**Statistical analysis**

All the experimental data were calculated and statistical analysis is done using Microsoft Office Excel 2010.

**RESULTS AND DISCUSSIONS**

**Antibacterial activity**

Natural products have been shown to be a potential source of anti-infective agents; the classic example being that of Penicillin and Tetracycline. Flavonoids are a class of natural compounds possessing a wide range of pharmacological activities 19. Screening of herbal medicines has led to the discovery of new medicinal drugs which have efficient protection and treatment roles against various diseases 20. Results obtained in the present study revealed that the tested *I. frutescens* root EtOAc extract possessed potential antibacterial activity against pathogenic bacteria, *S. flexneri* 16, *S. dysenteriae* 1, *V. cholerae* non.O139(L4), *V. cholerae* non.O139(CSK6669), *S. pneumoniae* and *E.coli* (Table 1). The crude root extract of *I. frutescens* showed significant antibacterial activity against *S. dysenteriae* 1, *S. pneumoniae* around 11-14 mm. The highest antibacterial activity of 14 mm zone of inhibition in *S. dysenteriae* and least activity recorded in *S. flexneri* measured 8 mm at the concentration of 1mg/ml.

**Table 1: Zone of inhibition after applying root extracts on different bacterial strains. The ‘’ indicates no effect of extracts, whereas, the data showing zone of inhibition in mm ±SD (disc radius 5 mm).**

<table>
<thead>
<tr>
<th>Microorganisms used for assay.</th>
<th>Zone of Inhibition after 24 hours incubation. (mm in 3 repeats ±SD)</th>
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<tbody>
<tr>
<td></td>
<td>100 μg/disc</td>
</tr>
<tr>
<td><strong>S. flexneri</strong> 16</td>
<td>-</td>
</tr>
<tr>
<td><strong>S. dysenteriae</strong> 1</td>
<td>6.5 ±0.707</td>
</tr>
<tr>
<td><strong>V. cholerae</strong> non.O139(L4)</td>
<td>6x 0</td>
</tr>
<tr>
<td><strong>V. cholerae</strong> non.O139(CSK6669)</td>
<td>-</td>
</tr>
<tr>
<td><strong>S. pneumoniae</strong></td>
<td>6x 0</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td>-</td>
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**DPPH radical scavenging activity**

Reactive oxygen species (ROS) are produced by all aerobic organisms and can easily react with most biological molecules including protein, lipids, lipoproteins and DNA. This ROS can damage the cell membranes and DNA and also cause protein and lipid peroxidation and other chemical reactions 21,22. Natural antioxidants can terminate or retard the oxidation process by scavenging free radicals. The reduction of methanol DPPH solution in presence of a hydrogen donating antioxidant is due to formation of nonradical form DPPH-H by the reaction. The extract was able to reduce the stable radical DPPH to the yellow coloured diphenyl picrylhydrazine 23. In this study of the ethyl acetate root extract at different concentrations is shown in Figure-1. It showed highest 51.48% of inhibition at the 75 μg/ ml of extract concentration, whereas, the positive control (L-ascorbic acid) gives much higher 73.96%% of inhibition at the same concentration. So the extract does not show the activity up to the positive control.
Superoxide anion scavenging activity

The percentage of inhibition of the extract on superoxide anions radicals determines the radical scavenging activity in *in vitro* methods. Superoxide anion is also very harmful to cellular components. It has been reported that flavonoids are effective antioxidants mainly because of scavenging efficacy of superoxide anions. In PMS/NADH-NBT system superoxide anion derived from dissolve oxygen by PMS/NADH coupling reaction reduces NBT. The decreasing absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture. The superoxide radical scavenging activities of the plant extract and the reference compound are increased markedly with increasing concentrations. Data shows that, highest percentage inhibition of root extract (84.348%) for the concentrations of 25 μg/ml and minimum inhibition (35.208%) in for concentration of 250 ng/ml. Figure 2 shows the bar diagram for percentage scavenging activity of root and extract. The negative control without extract shows zero (0) scavenging activity. As the BHA were taken as positive control, the scavenging activity between root extract and BHA and their comparison in bar diagram.

Figure 2: % of inhibition of superoxide radical scavenging activity between root extract and BHA and their comparison in bar diagram.

Alpha-glucosidase inhibitory assay

In type 2 diabetic mellitus inhibition of α-D glucosidase is beneficial. This enzyme converts carbohydrate into glucose. So inhibition of this enzyme will delay the absorption of glucose after meal and finally the glucose level will return into normal limit. The percentage of inhibition of root extract was calculated, in *α*-glucosidase inhibitory assay, one of the *in vitro* anti-diabetic activity determining methods. As per data, the ethyl acetate root extracts showed highest 81.012 % inhibition for the concentration of 500 μg/ml. In the concentration of 1 μg/ml of extract showed the minimum inhibition (9.448%). Figure 3 shows the bar diagram for *α*-glucosidase inhibitory assay of *I. frutescens* root extract. The negative control without extract shows zero (0) inhibition. The drug acarbose showed 90.47% inhibition in 500 μg/ml. This data showed that the percentage inhibition of acarbose is significantly higher than the percentage inhibition of root extract in same concentration of both.

Figure 3: % of inhibition of *α*-glucosidase inhibitory assay between root extract and acarbose.

CONCLUSION

The goal of this study was whether *Ichnocarpus frutescens* has any potent medicinal activity. We have studied antimicrobial, *in vitro* antioxidant and *in vitro* anti-diabetic activities and observed significant results. The preliminary results obtained from this study indicate that EtOAc extract of *I. frutescens* roots is not so effective in most of the tested pathogenic bacteria. But the antioxidant and anti-diabetic activities were significant. Further study in animal model to confirm the anti-diabetic activity of this extract is warranted. It may be true that the isolated pure chemicals from this crude extract may have better effect in all these activities but need to confirm in both *in vitro* and *in vivo* models in future.

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