Academic Sciences Asian Journal of Pharmaceutical and Clinical Research
Vol 6, Suppl 1, 2013 ISSN - 0974-2441

Research Article

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

ASHISH KUMAR SINGHA[1], BHASKAR BHATTACHARJEE[1], NIRANJAN DAS[2], BISWANATH DINDA[2], DEBASISH MAITI[*1]

[1]Department of Human Physiology, Tripura University, Suryamaninagar, Tripura, India 799 022, [2]Department of Chemistry, Tripura University, Suryamaninagar, Tripura, India. 799 022, Email: debumaiti@gmail.com

Received:8 November 2012, Revised and Accepted:14 December 2012

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 illness including fever, dyspepsia, skin troubles and headache. The possible antitumor activity and antioxidant 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 antidiabetic 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* non0139(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 antidiabetic 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, antioxidant and antidiabetic activities.

Keywords: *Ichnocarpus frutescens,* antibacterial, antidiabetic, antioxidant, 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 illness. This plant is also known as Dudhi; 'Shyamalata' in Bengali, 'Black creeper' in English and 'Ananta', 'Sariva' 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 [1]. 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 aceteminophen overdose, and correct hyperlipidemia in diabetic rats. It also has analgesic and anti-inflammatory properties, reduces fever, and lowers fasting glucose and improves glucose tolerance in diabetes [2].

Earlier works with chloroform and methanol extracts of this plant reported the antitumor activity and antioxidant role against Ehrlich ascites carcinoma (EAC) in mice[3], *in vitro* antitumor activity of polyphenolic extract of the leaves against U-937 and K-562 cell lines [4], antioxidant activity of ethyl acetate fraction of methanol extract of the flower[5] and isolation of sorboside from the aerial part [6]. 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 [7], hepatoprotective and antioxidant activity on paracetamol-induced hepatotoxicity in rats [8], screening for the antioxidant 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* [5], the wound healing potential on different experimental models of wounds in rats of the methanol roots extract [9], 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 [10], 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[11], the extract of *I. frutescence* leaves which were tested against streptozotocin-induced diabetic rats[12],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 [8]. It was also published that the hexane, chloroform and aqueous extracts from *I. frutescens* roots were evaluated for their antimicrobial activity [13].

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 antioxidant activity (*viz*. superoxide radical scavenging activity, DPPH radical scavenging activity) and *invitro* biochemical antidiabetic activity (*viz*. α-glucosidase inhibitory assay).

MATERIALS AND METHODS

Collection of plant species

The plant materials were collected from Jolaibari, Kalsi, 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 mess 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 vacuo to get a residue (104gm). 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, Strephylococcus aureus* and *Escherichia coli,* collected from National Institute Cholera and Enteric Disease, Kolkata and Agt. 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 [Duraipandiyan et al. 2006] on selected microorganisms with some modifications [14].

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 of 40 µl of each strain. After solidification of the agar, paper discs (5 mm in diameter and 0.4 mm in thickness), it 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 37^0 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), phenazonium methosulphate (PMS), p-nitrophenyl α-D-glucopyranoside (p-NPG), α-glucosidase, sodium carbonate (Na_2CO_3), 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 hydroxyl anisole (BHA) and methanol were obtained from MERCK 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[15]. 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 25^0C 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. Butylated hydroxyanisole (BHA) used as positive control [16], was dissolved in methanol as per each extract concentration.

$$\% \text{ of Inhibition} = \frac{\text{O.D. of Control} - \text{O.D. of Test}}{\text{O.D. of Control}} \times 100$$

DPPH radical scavenging activity

DPPH radical scavenging activity was measured according to the method of Cotelle *et al.* with some modification[15]. 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 -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 Na_2CO_3. 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.0139(L4), *V. cholerae* non.0139(CSK6669), *S. pneumonae* and *E.coli* (Table-1). The crude root extract of *I. frutescens* showed significant antibacterial activity against *S. dysentariae* 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* 16 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 radii= 5 mm).

Microorganisms used for assay.	Zone of Inhibition after 24 hours incubation. (mm in 3 repeats ±SD)		
	100 µg/disc	500 µg/disc	1000 µg/disc
***S. flexneri* 16**	-	6.5 ±0.19	8 ±0.433
S. dysenterae 1	6.5 ±0.707	8.5 ±0.707	14 ± 0
V. cholerae non.0139(L4)	6± 0	7.77±0.577	9.5 ±0.707
V. cholerae non.0139(CSK6669)	-	6.5 ±0.707	8.5 ± 0.19
S. pneumoniae	6± 0	7.5 ±0.707	11.5 ±0.707
E. coli	-	7± 0	9± 0

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 generate oxidative stress and produce many physiological disorders [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 51.484% of inhibition at the 75 µg/ ml of extract concentration, whereas, the positive control (L-ascorbic acid) gives much higher 73.969% of inhibition at the same concentration. So the extract does not show the activity up to the positive control.

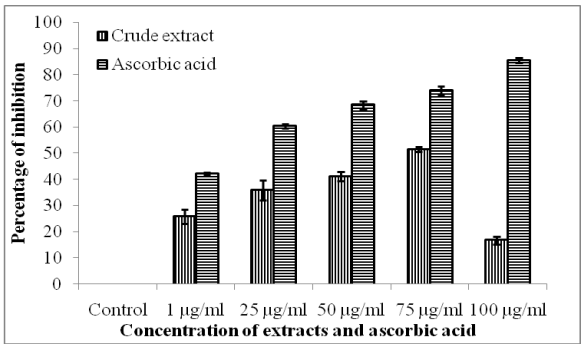

Figure 1: % of inhibition of DPPH radical scavenging activity between root extract and BHA and their comparison in bar diagram.

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 [24]. 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 [23]. 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 extract. The negative control without extract shows zero (0) scavenging activitity. As the BHA were taken as positive control, the maximum inhibition activity shows 15.345% in its 25 µg/ml. This data shows that the percentage inhibition of the root extract is significantly higher than the percentage inhibition of BHA in same concentration of both. It indicates that the ethyl acetate root extract has significant superoxide scavenging activity.

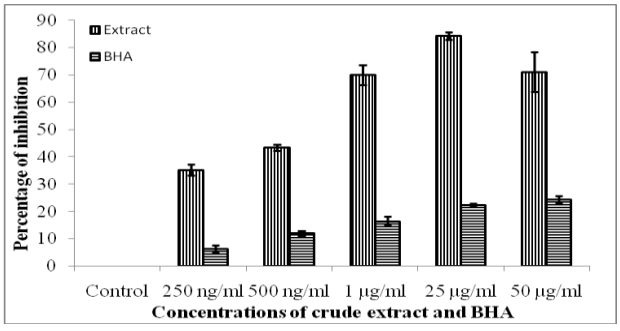

Figure 2: % of inhibition of superoxide radical scavenging activity between root extract and BHA and their comparison in bar diagram

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* antidiabetic 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 % inhibition of root extract in same concentration of both.

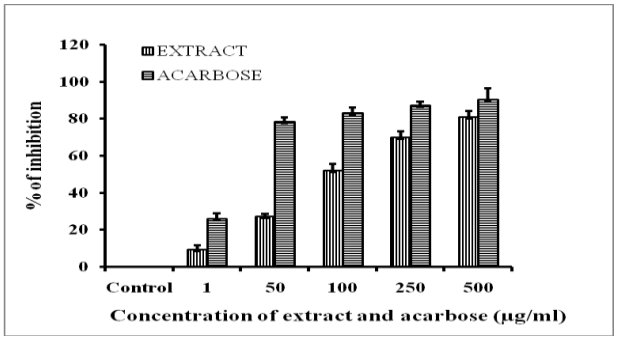

Figure 3: % of inhibition of α-glucosidase inhibitory assay between root extract and acarbose .

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.

ACKNOWLEDGEMENT

- Tripura University for providing fellowship
- DBT, Govt. of India, for funding.
- Dr. M. K. Chakraborty and Dr. A. Pal, NICED, Kolkata, for kind gift of pathogenic bacterial strains.
- Dept. of Microbiology, AGMC, Agartala, also for providing some pathogenic bacterial strains.

REFERENCES

1. Mishra A, Pradhan DK, Mishra MR, Kumar S, Meher A. Phytochemical screening of *Ichnocarpus Frutescens* plant parts. Int J Pharmacogn Phytochem Res 2009; 1(1): 5-7.
2. Singh N, Mani T, Prakash D, Singh P. A Review on Medicinal Properties of *Ichnocarpous frutescens*. Indian J of Novel Drug Delivery 2012; 4(1): 24-27.
3. Dash D K, Nayak S S, Samanta S, Ghosh T, Jha T, Maiti B C, Maity T K. Antitumor activity and Antioxidant Role of *Ichnocarpus frutescens* Against Ehrlich Ascites Sarcinoma in Swiss Albino Mice. Natural Product Sciences 2007; 13(1): 54-60.
4. Kumarappan C T, Mandal S C. Antitumor Activity of Polyphenolic Extract of *Ichnocarpus frutescens.* Exp Oncol 2007; 29(2): 94-101.
5. Patwekar FI, Patwekar MF, Asif M, Heroor S, Mohsin AM. Activity guided separation of phytoconstituents from the flowers of *Ichnocarpus frutescens* L. and evaluation for antioxidant property. Res J Pharma Biological and Chem Sciences 2010; 1(4): 318-23.
6. Verma R K, Gupta M M; A New Sorboside From *Ichnocarpus frutescens,* Indian J of Chem 1988; 27B: 283-284.
7. Barik R, Jain S, Qwatra D, Joshi A, Tripathi GS, Goyal R. Anti-diabetic activity of aqueous root extract of *Ichnocarpus frutescens* in streptozotocin-nicotinamide induced type-II diabetes in rats. Indian J Pharmacol 2008 ;40: 19-22.
8. Dash DK, Yeligar VC, Siva SN, Tirtha G, Rajalingam D, Pinaki S *et al* . Evaluation of hepatoprotective and antioxidant activity of *Ichnocarpus frutescens* (Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. Tropical J Pharma Res 2007; 6(3): 755-65.
9. Pandurangan A, Khosa RL, Hemalatha S. Evaluation of wound healing activity of *Ichnocarpus frutescens* root. Der Pharmacia Letter 2010; 2(3): 444-49.

10. Pandurangan A, Khosa RL and Hemalatha S. Evaluation of anti-inflammatory and antioxidant activity of *Ichnocarpus frutescens* root. DARU 2009; 17(1): 1-5.
11. Pandurangan A, Khosa RL and Hemalatha S. Evaluation of anti-pyretic potential of *Ichnocarpus frutescens* roots. Int J Pharmacol Therap 2009; 8(1): 47-50.
12. Subash-Babu P, Ignacimuthu S and Agastian P. Insulin secretagogue effect of *Ichnocarpus frutescence* leaf extract in experimental diabetes: a dose-dependent study. Chem Biol Interact 2008; 172(2): 159-71.
13. Malathy NS, Sini S. Antimicrobial activities of *Ichnocarpus frutescens* (L.) R.Br. and *Hemidesmus indicus* R.Br. Roots. Ancient science of Life 2009; 28(4): 13-15.
14. Duraipandiyan V, Ayyanar M, Ignacimuthu S. Antimicrobial activity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. BMC Complementary and Alternative Medicine 2006; 6**:** 35.
15. Mondal S K, Chakraborty G, Gupta M, Mazumdar U K. In vitro Antioxidant Activity of *Diospyros malabarica* Kostel bark. Indian J of Exp Biol 2006; 44: 39-44.
16. Ogunlana O E, Ogunlana O O. In vitro Assessment of the Free Radical Scavenging Activity of *Psidium guajava*. Res J of Agri and Bio Sciences 2008; 4(6): 666-671.
17. Gupta R C, Sharma V, Sharma N, Kumar N, Singh B. In vitro Antioxidant Activity from Leaves of *Oroxylum indicum* (L.) Vent. A North Indian Highly Threatened and Vulnerable Medicinal Plant. J of Pharm Res 2008; 1(1): 65-72.
18. Yin J, Heo SI1, Wang M H. Antioxidant and Antidiabetic Activities of Extracts from *Cirsium japonicum* roots. Nutrition Research and Practice 2008; 2(4): 247-251.
19. Jayashree N, Narayanan N, Lata Sriram. Antibacterial, antifungal and anti-mycobacterial studies on some synthetic dimethoxy flavones. Asian J Pharm Clin Res 2012; 5(1): 101-103.
20. Chinsembu KC, Hedimbi M. Ethnomedicinal plants and other natural products with

anti-HIV active compounds and their putative modes of action, Int J Biotech and Mol Biol Res 2010; 1(6): 74-91.

21. Kourounakis AP, Galanakis DK. Synthesis and pharmacological evaluation of novel derivatives of anti-inflammatory drugs with increased antioxidant and anti-inflammatory activities. Drug Development Research 1999; 47: 9-16.
22. Gulcin I, Buyukokuroglu ME. On the in vitro antioxidant properties of melatonin. J Pineal Res 2002b; 33: 167-171.
23. Mandal P, Mishra TK, Ghosal M. Free-radical scavenging activity and phytochemical analysis in the leaf and stem of *Drymaria diandra* Blume. Int J Integrative Biol 2009; 7(2): 80-84.
24. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem Pharmacol 1988; 37(5): 837-41.