International Journal of PharmTech Research
CODEN (USA): IJPRIF, ISSN: 0974-4304
Vol.8, No.5, pp 843-847, 2015

Phytochemical Analysis, Anti-Arthritic and Anti-Diabetic Activities of the Leaf Extracts of Ipomoea eriocarpa

Moonjit Das, Himaja Malipeddi*

[1]Pharmaceutical Chemistry Division, School of Advanced Sciences, VIT University, Vellore - 632014, Tamil Nadu, India

Abstract: *Ipomoea eriocarpa* belongs to the Convolvulaceae family and has a reputation as a folk medicine in the treatment of headache, rheumatism, leprosy, epilepsy and ulcers although there is no scientific evidence till date. The dried and powdered leaves of *I. eriocarpa* were extracted with water, chloroform and petroleum ether by Soxhlet method and preliminary phytochemical analysis of three extracts was carried out. All the three extracts were screened for the *in vitro* anti-arthritic and anti-diabetic activity. The preliminary phytochemical analysis of the three extracts revealed the presence of alkaloids, phenols, saponins, phytosterols, flavanoids and terpenoids. The extracts of *I. eriocarpa* have shown significant anti-arthritic activity against protein denaturation. The chloroform and petroleum ether extracts have shown potent α-amylase enzyme inhibitory activity as compared to water extract, probably due to the presence of terpenoids, which are absent in water extract.
Keywords: *Ipomoea eriocarpa*, phytochemical analysis, anti-inflammatory, anti-arthritic, anti-diabetic.

Introduction and Experimental

Folk medicines have played a very important role in health care needs of India and other countries for a long period of time. Recently traditional Indian drugs have gained a lot of popularity in the international medical and pharmaceutical institutions as a valuable source of medicinal agents. Over thousands of years, the Indian tradition and herbal drug products have been used in the treatment of various human disorders. However, scientific data on their efficacy, pharmacological properties, mechanism of action and their chemical constituents have so far been lacking.

Ipomoea eriocarpa is a summer annual or perennial broadleaf plant belonging to the Convolvulaceae family. This plant is distributed throughout the African and South American tropical regions, tropical Asia and Northern Australia. They are slender twining herb of grassland, waste spaces and a weed of cultivation. The plant is traditionally used as a vegetable in India for its tender leaves and stems. The seeds are nutritious and a good source of carbohydrates and proteins. The plant has many unspecified medicinal use in India. The oil extract of the plant is used treatment of headache, rheumatism, leprosy, epilepsy, ulcers and fevers.[1] Scientific investigation of *Ipomoea eriocarpa* recently demonstrated cerebroprotective,[2] antioxidant,[3] antisecretory,[4] antinociceptive,[5] antipyretic,[6] toxicity,[7] anthelmintic and insecticidal activity.[8]

In the present study, phytochemical analysis of the three extracts (water, chloroform and petroleum ether) as well as protein denaturation inhibitory assay (anti-arthritic) and α-amylase inhibition assay (anti-diabetic) of the three extracts of the leaves of the plant have been assessed. This is the first report of the *in vitro* anti-arthritic and anti-diabetic activity of the plant.

Plant material

The leaves of *I. eriocarpa* were collected from Kamrup and Udalguri district of Assam, India. The plant was identified as *I. eriocarpa* at the Department of Botany, Gauhati University, Assam, India. The voucher specimen of the plant was deposited at the college for further reference.

Proximate analysis

Proximate analysis of the leaves of *I. eriocarpa* like determination of total ash value, acid insoluble ash value, water soluble ash value, alcohol and water soluble extractive value and moisture content were performed by standard procedures.[9]

Preparation of extracts

Dried powder of the leaves of *I. eriocarpa* were subjected to continuous hot extraction in a Soxhlet apparatus using three solvents namely water, chloroform and petroleum ether. The solvent was removed by distillation under reduced pressure, which produced a greenish sticky residue. The concentrated crude extract was stored at 4°C in a refrigerator and used for the further studies.

Preliminary phytochemical screening

A small portion of the dry extracts was subjected to systematic phytochemical screening for the presence of chemical constituents like carbohydrates, alkaloids, phenols, glycosides, saponins, flavanoids, phytosterols, terpenoids and proteins.[10, 11]

Protein denaturation inhibitory assay (Anti-arthritic Activity)

The anti-arthritic activity of the extracts was estimated by protein denaturation method with some modifications.[12] The reaction mixtures (5 ml) consisted of 0.2 ml of egg albumin (from fresh hen's egg), 2.8 ml of phosphate buffered saline (pH 6.4) and 2 ml of varying concentrations of aqueous solution of the three extracts of *Ipomoea eriocarpa* so that the final concentration is 50-200 µg/ml. Similar volume of distilled water served as control. Then the mixtures were incubated at 37°C in a water bath for 15 minutes and then heated at 70°C for 5 minutes. After cooling, their absorbance was measured at 660 nm. Diclofenac sodium (50-200µg/ml) was used as a reference drug. All The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\text{Inhibition (\%)} = 100 \times \frac{A_t}{1 - A_c}$$

Where,
A_t = Absorbance of test sample and A_c = Absorbance of control.

α-Amylase enzyme inhibitory assay (Anti-diabetic activity)

α-Amylase enzyme inhibitory activity of the plant extracts in hydrolysis of starch was studied using as standard.[13] The extracts of varying concentrations (50-200 µg/ml) were pre-incubated with α-amylase enzyme solution (5U/ml) for 20 minutes. 0.5% starch solution in 20 mM phosphate buffer was added to the reaction mixture and incubated at 370°C in addition for 20 minutes. 2,4-dinitro-salicylic acid reagent was added to the reaction mixture and maintained at 100°C for 15 minutes to end the catalytic activity. The samples were diluted and the absorbance was measured at 540 nm using Hitachi U-2910 Spectrophotometer. The percentage inhibition for the samples and control were calculated from the mean absorption by the following formula:

$$\text{α-Amylase inhibition (\%)} = 100 \times \frac{A_c - A_t}{A_c}$$

Where,
A_t = Absorbance of test sample and A_c = Absorbance of control.

Statistical Analysis

All tests were conducted in triplicate. Data are reported as means ± standard deviation (SD). Results were analyzed statically by using Microsoft Office Excel 2013 (Roselle, IL, USA).

Results and Discussion

Proximate analysis

The leaves of *I. eriocarpa* was subjected to evaluate its total ash value, acid insoluble ash, water soluble ash (Table 1), water soluble extractive value, alcohol soluble extractive value (Table 2) and moisture content (Table 3).

Table 1. Ash value of *Ipomoea eriocarpa* leaf

Ash value		
Total	**Acid insoluble**	**Water soluble**
9.75%	5.56%	4.18%

Table 2. Extractive value of *Ipomoea eriocarpa* leaf

Extractive value (Percentage w/w)	
Alcohol soluble	**Water soluble**
12.38%	8.76%

Table 3. Moisture content of *Ipomoea eriocarpa* leaf

Time (minutes)	Moisture content (%)
30	13.68
45	10.45
60	7.63
75	6.46
90	5.98

Preliminary phytochemical screening

The three extracts obtained from the leaves of *I. eriocarpa* were tested for various chemical constituents and the results obtained are given in Table 4.

Table 4. Phytochemical screening of *Ipomoea eriocarpa* leaf extracts

Chemical Constituents	Water Extract	Chloroform Extract	Petroleum Ether Extract
Alkaloids	+	+	+
Carbohydrates	-	-	-
Glycosides	-	-	-
Saponins	-	+	-
Phytosterols	+	+	+
Flavanoids	-	-	-
Phenols	+	+	+
Proteins	-	-	-
Terpenoids	-	+	+

+ = Present, - = Absent

Protein denaturation inhibitory assay (Anti-arthritic Activity)

The effect of the three extracts of *I. eriocarpa* was evaluated against denaturation of egg albumin. The results are summarized in Figure 1. The presenting findings exhibited a concentration dependent inhibition of protein denaturation by the extracts of *I. eriocarpa* throughout the concentration range of 50-200 µg/ml. Diclofenac sodium at concentration 200 µg/ml showed the highest effect of 91%. Among the three extracts, water and chloroform extract showed the maximum effect of about 80% at the concentration of 200 µg/ml.

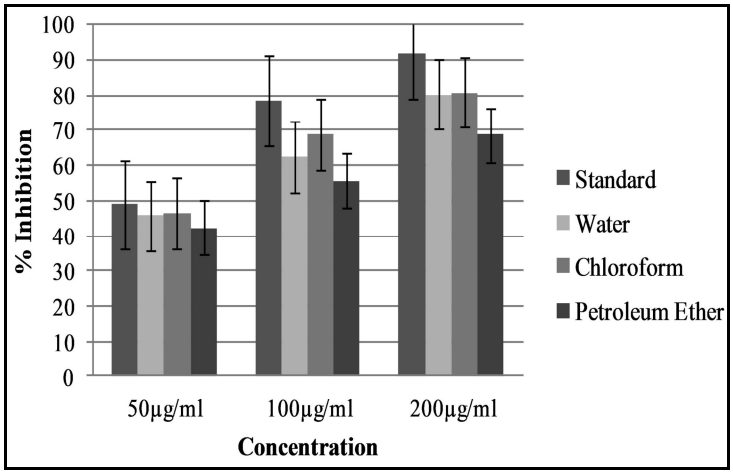

Figure 1. Protein denaturation inhibitory assay of *Ipomoea eriocarpa* leaf extracts. The data represent the mean ± standard deviation of triplicate experiments.

α-Amylase enzyme inhibitory assay (Anti-diabetic activity)

α-Amylase enzyme is involved in breaking down of complex starch in to simpler glucose units play an important role in maintaining the blood glucose level. The assay results are summarized in Figure 2. The petroleum ether extracts showed similar activity as that of the standard drug. At 200 µg/ml, both the petroleum ether extract and the standard showed the highest effect of about 75%. However, the water extract possessed less effect against α-amylase enzyme inhibition, probably due to the presence of terpenoids.

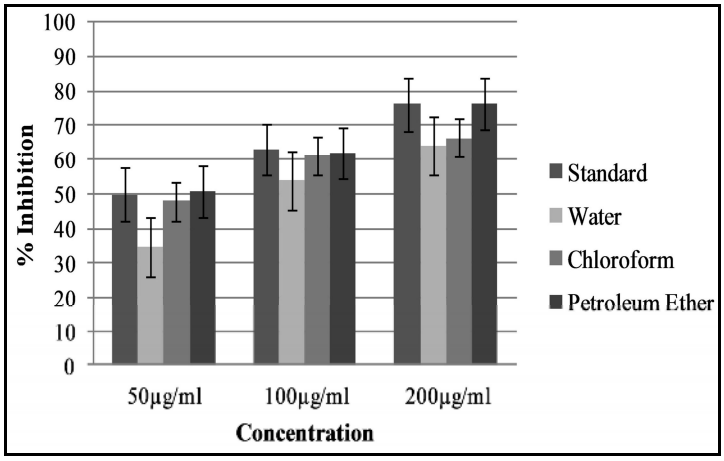

Figure 2. α-Amylase enzyme inhibition assay of *Ipomoea eriocarpa* leaf extracts. The data represent the mean ± standard deviation of triplicate experiments.

Conclusions

The results suggested that the preliminary phytochemical analysis revealed the presence of alkaloids, phenols and phytosterols in all the three extracts, terpenoids in the chloroform and petroleum ether extracts and additionally saponins in the chloroform extract. The extracts of *I. eriocarpa* have significant anti-arthritic effect against the denaturation of protein. The chloroform and petroleum ether extracts have shown potent α-amylase enzyme inhibition activity as compared to water extract, probably due to the presence of terpenoids, which are absent in water extract. However, isolation and characterization of the chemical compounds responsible for these activities as well as *in vivo* studies need to be performed to further confirm these observations. The major phytoconstituents from the plant are currently being isolated.

Acknowledgements

We are grateful to VIT University, Vellore for providing the research facilities.

References

1. Madhava C.K., *Ipomoea eriocarpa*: Chittoor medicinal plants, Tirupati: Himalaya Book Publications, 2005.
2. Vijayakumar S., Sasikala M. and Dhanapal, R., Cerebroprotective effect of *Ipomoea eriocarpa* against cerebral ischemia in rats, Int. J. Exp. Pharmacol., 2012, 2, 13-18.
3. Thamizhvanan K., Kumuda P. and Sateesh Babu J.M., Antioxidant activity of whole plant of *Ipomoea eriocarpa* extract, Int. J. Innov. Drug. Discover., 2012, 2, 1-3.
4. Senthil Kumar K.K., Evaluation of antisecretory activity of *Ipomoea eriocarpa* in albino wistar rats, *Int. J. Exp. Pharmacol.*, 2012, 2, 19-25.
5. Prasad P.S., Mantry S., Reddy G.R. and Jaffer Sadik M.D., Antinociceptive activity of *Ipomoea eriocarpa* whole plant extracts, Int. J. Res. Pharmacol. Pharm., 2012, 1, 85-88.
6. Naik N.D., Reddy R.G., Naadella B.C. and Suresh B., A study on antipyretic activity of petroleum ether extract of *Ipomoea eriocarpa* whole plant, Int. J. Pharmacol. Screen. Methods, 2012, 2, 58-60.
7. Babu B.H., Toxicity studies on *Ipomoea eriocarpa* extract in experimental animals, Int. J. Pharm. Sci. Res., 2012, 2, 7-11.
8. Das M. and Himaja M., Phytochemical screening, GC-MS analysis and biological activity of *Ipomoea eriocarpa* leaf extracts, Int. J. Pharm. Pharm. Sci., 2014, 6, 592-594.
9. Tanaka T., Nonaka G. and Nishioka I., Punicafolin, an ellagitannin from the leaves of *Punica granatum*, Phytochem., 1985, 24, 2075-2078.
10. Trease G.E. and Evans W.C., Textbook of pharmacognosy, London: Balliere Tindall, 1989.
11. Harborne J., Phytochemical methods: A guide to modern techniques of plant analysis, London: Chapman & Hall, 1998.
12. Grant N.H., Alburn H.E. and Kryzanauskas C., Stabilization of serum albumin by anti-inflammatory drugs, Biochem. Pharm., 1970, 19, 715-722.
13. Mogale M.A., Lebelo S.L., Thovhogi N., De Freitas A.N. and Shai, L.J., α-Amylase and α-glucosidase inhibitory effects of *Sclerocarya birrea* [(A. Rich.) Hochst.] subspecies *caffra* (Sond) Kokwaro (Anacardiaceae) stem-bark extracts, African J. Biotech., 2011, 10, 15033-15039.

*****