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SHORT COMMUNICATION

PHYTOCHEMICAL AND ACUTE TOXICITY STUDY ON *BRYOPHYLLUM CALYCINUM* SALISB.

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Keywords: *Bryophyllum calycinum* Salisb. (Crassulaceae), phytochemical investigation, acute toxicity study.

The plant *Bryophyllum calycinum* Salisb. (Crassulaceae) is a shrub mainly found in the tropical parts of Bengal and in southern Africa and American continents. It is locally known as "Patharkuchi" and for long has been used in Ayurvedic medicine. As per traditional use, the leaves and leaf juice are used as antiviral, antipyretic, antimicrobial, anti-inflammatory, antitumorous, hypocholesterolemic, antioxidant, diuretic, antiulcer, styptic, antidiabetic, astringent, antiseptic, antilithic and cough suppressant (1-9). Some activities of some plants of this genus have been reported (10-12). The present study was undertaken to report the phytoconstituents present in successive extracts of the leaves of the plant, to determine the ash values and extractive values of the dried leaves and to find out the LD_{50} values of methanolic and aqueous extracts of the leaves of the plant.

EXPERIMENTAL

Plant material

The plant was identified (Ref. No. CNH/I-I(53)/2004-Tech-I/885) by the taxonomists of Botanical Survey of India, Shibpur, Howrah. After authentication, the fresh leaves were collected in bulk from young matured plants at the rural areas of Howrah during August – September 2005 and washed, shade dried and milled into coarse powder by a mechanical grinder. The powder was passed through sieve number 40 (B.P. standard) and used for further studies.

Preparation of extracts

The powdered plant material was extracted successively with analytical grade, redistilled petroleum ether (40-60°C), chloroform and methanol (procured from S.B. Fine Chem. Ltd., Mumbai and Merck, Mumbai, respectively) using Soxhlet apparatus. The solvents were removed under reduced pressure to obtain greenish-black (PE), brownish-black (CE) and blackish-brown (ME) colored solid residues (yield 3%, 1.8% and 5.7% w/w on dried plant material basis, respectively). The aqueous extract was prepared by decoction process using double distilled water and then it was filtered, evaporated and dried under reduced pressure to yield solid residue (AE) with a yield of 21% w/w on dried plant material basis. Phytochemical investigations were performed on all the four extracts and LD_{50} study was done on methanolic and aqueous extracts only in mice and rats.

Preliminary phytochemical analysis

The extracts prepared in different solvents were taken and standard methods were used to detect the nature of phytoconstituents present in them (13-16).

Determination of total ash

About 2-3 g accurately weighed powdered drug was incinerated in a silica dish at a temperature not exceeding 450°C until free from carbon. It was then cooled and weighed. The % w/w of ash with reference to the air-dried drug was calculated (17).

Determination of alcohol soluble extractive

Accurately weighed 5 g of air-dried powdered drug was macerated with 100 mL of alcohol of the specified strength in a closed flask for 24 h, shaken frequently during first 6 h and allowed to stand for 18 h. It was then filtered rapidly, taking precautions

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against loss of the solvent and 25 mL of the filtrate were evaporated to dryness in a tared flat-bottomed shallow dish and dried at 100°C to constant weight. The % w/w of alcohol soluble extractive value was calculated with reference to the air-dried drug (17).

Determination of water soluble extractive

Procedure was the same as for alcohol soluble extractive using water instead of ethanol.

Determination of sodium, potassium and calcium

An accurately weighed amount of the ash of the plant was digested with 5 mL of 10% HCl and filtered through Whatmann No. 41 filter paper. The residue was washed with hot water, cooled and made to volume. The sample solution was then compared in the flame photometer against standard solutions of NaCl, KCl and $CaCO_3$ containing the same amount of HCl. The concentrations of the sodium, potassium and calcium ions were calculated by extrapolation method (18).

Ethical clearance

The protocol used in this study for the use of animals was approved by the University Animal Ethical Committee.

Acute toxicity study

Swiss albino male mice (weighing 20-25 g) and rats (weighing 110-130 g) were administered intraperitoneally with graded doses (350-2600 mg/kg of body weight) of methanolic and aqueous extracts of the plant. After administration of the extracts the animals were observed for toxic effects during 24 h after the treatment. The toxicological effects were observed in terms of mortality and expressed as LD_{50}. The number of animals dying during the period was noted. The LD_{50} of the extracts were calculated by the method of Miller and Tainter (19, 20).

The methanolic and aqueous extracts were also administered orally in graded doses (500-3000 mg/kg body weight) in mice and rats to test their oral toxicity.

RESULTS AND DISCUSSION

The findings of the preliminary phytochemical investigations and the results of the LD_{50} study were depicted in the respective Tables. The preliminary phytochemical tests performed were of qualitative type and from the phytochemical investigations it was observed that alkaloids and tannins were pres-

Table 1. Qualitative phytochemical evaluation of the *Bryophyllum calycinum* Salisb. extracts.

Constituents	Observations*			
	PE	CE	ME	AE
Alkaloids	+	+	+	+
Flavonoids	+	-	+	-
Tannins	+	+	+	+
Sugars	-	-	+	+
Steroids	+	-	+	-
Organic acids	+	-	+	+

* + present, – absent.

Table 2. Determination of ash and extractive values of the *Bryophyllum calycinum* Salisb.

Ash value (%w/w)			Extractive value (%w/w)	
Total ash	Water soluble ash	Acid insoluble ash	Water soluble	Alcohol soluble
5.01	4.19	1.69	19.80	5.60

Table 3. Determination of calcium, potassium and sodium levels of *Bryophyllum calycinum* Salisb.

Calcium	Potassium	Sodium
96.45	76.40	UD

Unit : µg/g of crude drug. UD : Undetectable.

Table 4. Determination of LD_{50} values by Miller and Tainter method of the methanolic and aqueous extracts of *Bryophyllum calycinum* Salisb. in mice.

Group No.	Dose (mg/kg *i.p.*)	Log dose	Dead/ total	% Dead	Corrected %*	Probit	LD_{50} value from graph (mg/kg)
ME-1	400	2.6000	0/10	0	2.5	3.04	1159.03
ME-2	800	2.9000	2/10	20	20	4.16	
ME-3	1200	3.0790	4/10	40	40	4.75	
ME-4	1600	3.2040	6/10	60	60	5.25	
ME-5	2000	3.3010	9/10	90	90	6.28	
ME-6	2400	3.3800	10/10	100	97.5	6.96	
AE-1	350	2.4700	0/10	0	2.5	3.04	957.02
AE-2	650	2.7700	1/10	10	10	3.72	
AE-3	950	2.9500	3/10	30	30	4.48	
AE-4	1250	3.0700	5/10	50	50	5.00	
AE-5	1550	3.1700	8/10	80	80	5.84	
AE-6	1850	3.2500	10/10	100	97.5	6.96	

* Corrected formula : For the 0% dead: 100 (0.25/n). For the 100% dead: 100 [(n-0.25)/n], where n = number of animals in each group.

Table 5. Determination of LD_{50} values by Miller and Tainter method of the methanolic and aqueous extracts of *Bryophyllum calycinum* Salisb. in rat.

Group No.	Dose (mg/kg *i.p.*)	Log dose	Dead/ total	% Dead	Corrected %*	Probit	LD_{50} value from graph (mg/kg)
ME-1	600	2.7700	0/10	0	2.5	3.04	1459.69
ME-2	1000	3.000	1/10	10	10	3.72	
ME-3	1400	3.1400	4/10	40	40	4.48	
ME-4	1800	3.2500	6/10	60	60	5.25	
ME-5	2200	3.3400	8/10	80	80	5.84	
ME-6	2600	3.4100	10/10	100	97.5	6.96	
AE-1	500	2.6980	0/10	0	2.5	3.04	1064.21
AE-2	800	2.9000	2/10	20	20	3.72	
AE-3	1100	3.0400	5/10	50	50	4.16	
AE-4	1400	3.1460	7/10	70	70	5.00	
AE-5	1700	3.2300	9/10	90	90	5.84	
AE-6	2000	3.3000	10/10	100	97.5	6.96	

* Corrected formula: For the 0% dead: 100 (0.25/n). For the 100% dead: 100 [(n-0.25)/n], where n = number of animals in each group.

ent in all the extracts. Flavonoids and steroids were present in PE and ME. Sugars and organic acids were present in ME and AE. The total ash of the leaf powder was 5.01% w/w. The water and alcohol soluble extractive values were 19.80 and 5.60% w/w, respectively. The amount of calcium and potassium present in the total ash were 96.45 and 76.40 µg/g of crude drug, respectively. The aim of the total ash determination is to check the authenticity, purity and quality of the plant used for the study for reproducibility of the experimental results.

From the acute toxicity study it was observed that the LD_{50} values of ME in mice and rats were found to be 1159.03 and 1459.69 mg/kg, respectively and of AE were found to be 957.02 and 1064.21 mg/kg, respectively. The extracts were found to be

non-toxic orally in doses up to 3 g/kg body weight in mice and rats.

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REFERENCES

1. Bershtein E.I.: Vestnik Khirurgii Imeni I.I. Grekova 107, 116 (1972)..
2. Biberstein H.: Zentralbl. Haut Geschlechtskr. Grenzgeb. 22, 19 (1927).
3. Foulsham Gaind K.N. et al.: Planta Med. 23, 149 (1976).
4. Huang K.C.: The Pharmacology of Chinese Herbs., CRC Press, Boca Raton, Florida 1993.
5. Nadkarni A.K.: "The Indian Materia Medica", vol. I, 3rd Ed., pp. 716-717, Popular Prakashan, Bombay 1988.
6. Chopra R.N., Nayer S.L., Chopra I.C.: "Glossary of Indian Medicinal Plants", p. 147, Council of Scientific and Industrial Research, New Delhi 1992.
7. Asolkar L.V., Kakkar K.K.,Chakre O..J.: Second supplement to Glossary of Indian Medicinal Plants with Active Principles, part I (A-K), p. 382, Council of Scientific and Industrial Research, New Delhi 1992.
8. Da Silva S.A., et al.: Acta Trop. 60, 201 (1995).
9. Matos F.J.A. et al.: Plantas da Medicina Popular do Ceara Selecionadas pela Maior Frequencia de Seu Uso, p. 24, Vlll Simposio de Plantas Medicinais do Brazil, 4-6 Sept. 1984.
10. Rastogi R.P., Mehrotra B.N.: "Compendium of Indian Medicinal Plants", vol. 1, p. 63, PID, New Delhi 1960-1969.
11. Rastogi R.P., Mehrotra B.N.: "Compendium of Indian Medicinal Plants", vol. 2, pp. 112-401, PID, New Delhi 1970-1979.
12. Rastogi R.P., Mehrotra B.N.: "Compendium of Indian Medicinal Plants", vol. 3, p. 373, PID, New Delhi 1980-1984.
13. Trease G.E., Evans W.C.: "Pharmacognosy", 13th Edition, p. 171, ELBS Publication, Delhi 1989.
14. Harborne J.J.: "Phytochemical Methods: A guide to modern techniques of plant analysis", 2nd Ed., p. 85, Chapman and Hall, New York 1984.
15. Kokate C.K., Purohit A.P., Gokhale S.B.: "Pharmacognosy", 23rd Ed., pp. 106-114, Nirali Prakashan, Pune, 1998.
16. Khandelwal K.R.: "Practical Pharmacognosy: Techniques and Experiments", 13th Ed., pp. 149-156, Nirali Prakashan, Pune 2005.
17. Pharmacopoeia of India, Ministry of Health and Family Welfare, Appendix 3, pp. A-53-54, Government of India, vol. II, New Delhi 1996.
18. Jeffery G.H., Bassett J., Mendham J., Denney R.C.: "Vogel's textbook of quantitative chemical analysis" 5th Ed., pp. 797-798, Longman Ltd, London 1989.
19. Ghosh M.N.: "Fundamentals of Experimental Pharmacology", 2nd Ed., pp. 177-190, Scientific Book Agency, Calcutta 1984.
20. Rajeshwer Y, Gupta M, Mazumder U.K.: Iranian J. Pharmacol. Ther. 4, 46 (2005).

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