Acta Pharmaceutica Turcica
46: 149-159 (2004)

Antidiarrhoeal Activity of *Argyreia speciosa* Flower: an Ethnopharmacological Study

Ch. V. Rao*, S.K.Ojha, G.D. Reddy, A.K.S. Rawat, G.M.M. Rao and P.Pushpangadan

Ethnopharmacology Division, National Botanical Research Institute, (Council of Scientific and Industrial Research) Rana Pratap Marg, Post Box No. 436, Lucknow-226 001, Uttar Pradesh, INDIA.

Abstract

Diarrhoea is a wide spread disease that is often reported to the physicians for treatment, in addition to countless instances where diarrhoea is treated by folklore medicine. *Argyreia speciosa* Sweet (Convulvulaceae) commonly known as Elephant creepr. The 50% ethanolic flower extract of *A. speciosa* was undertaken to evaluate the anti-diarrhoeal activity against validated experimental models of diarrhoea in rats. The flower extract has standardised by HPTLC analysis in the Toluene: Ethylacetate: Formic acid (5: 5: 1) and resolved peaks were visualised at 336nm with a 400 k filter at 0.52, 0.60, 0.70 and 0.77 Rf. The λ max for major spots was 332, 320, 288 and 291 respectively. The densitometric absorption at 200nm was 31.29%, 27.79%, 22.06% and 14.89%. Administration of extract 50- 150 $mgkg^{-1}$ orally showed dose dependent decrease in the intestinal propulsion from 61.54% - 41.36%, which is equivalent to 38.46% - 58.64% intestinal propulsive inhibition relative to control 27.23%. Further study on castor oil induced diarrhoea and intestinal fluid accumulation causes a dose dependent decrease in the number of faecal matter and a significant reduction in the intestinal fluid accumulation from 9.97% - 39.58%. The results suggest that flower extract of *A. speciosa* had significant antidiarrhoeal activity.

Keywords: *Argyreia speciosa* Sweet, diarrhoea, flowers.

Introduction

Diarrhoea continues to be one of the leading causes of mortality and morbidity especially in children in developing countries including India (Black *et al.*, 1982) and the cause of 4 –5 million deaths throughout the world annually (Mukherjee *et al.*, 1998). From the vast array of the Materia Medica of the indigenous system, plants have been reported to have activity against diaarhoea and act as very useful remedies for the alleviation of the human suffering. The world health organisation (WHO) has constituted a diarrhoeal disease control programme (CDD), which includes the study of traditional medical practices, together with the elevation of the health education and prevention approaches (Anonymous, 1979; Syder and Merson 1982; Lutterodt, 1989). *Argyreia speciosa* Sweet (Convolvulaceae) commonly known as "Elephant Creeper" in English and "Samandar-Ka Pat" in Hindi and Vidhara or Vriddhadaruka in Ayurveda, is a large woody climber, rarely suberect shrub. The deep purple coloured flower which open during the rainy season, are arranged in subcapitate cymes, peduncles 3 - 6 inches long; bract large, foliaceous, ovate, acuminate, deciduous, pedicels and calyx white – tementose. Corolla 2 – 2.5 inch, long tubular funnel – shaped, the bands silky – pubescent

* *Corresponding author: E-mail: chvrao72@yahoo.com*

outside, limb 2 inch in diameter (Duthie, 1960). *A. speciosa* is a native of Bengal and found throughout India except in dry, western region up to 1000 ft. elevation and is often cultivated (Kapoor, 2000).

The roots of *A. speciosa* are reported to be tonic, bitter, aphrodisiac, diuretic and used in rheumatism, gonorrhoea, chronic ulcers and disease of nervous system (Anonymous, 1988; Chopra, *et al.*, 1956). Seed oil has antifungal activity (Mishra and Chaturvedi, 1978). Leaves are extensively used all over India in the treatment of ulcers, boils, carbuncles and tumours with benefit of great extent (Chopra, *et al.*, 1956). Leaves are reported to be effective in diabetes (Bhargava and Singh., 1978; Chao and Marderosian, 1973; Desai, *et al.*, 1975). The flower bud is used in chronic diarrhoea and dysentery (Jain and Tarafder, 1970) but scientific reports are anecdotal. Earlier reports from our laboratory showed the antidiarrhoeal, analgesic and antiulcer activity of *Cissampelos pareira* root (Rao *et al.*, 2004), fruits of *Aegle marmelos* (Rao *et al.*, 2003) in experimental animals. The flower of *Argyreia speciosa* has long been used by the quacks in treatment of diarrhoea. In continuation of our studies we subjected the aqueous ethanolic extract of the flowers of *Argyreia speciosa* in experimental animals.

Materials and Methods

Plant material: The fresh flowers of *A. speciosa* (Family: Convolvulaceae) were plucked from trees found in the campus garden of National Botanical Research Institute, Lucknow, India in September 2002. The plant material was identified and authenticated taxonomically at National Botanical Research Institute, Lucknow, India. A voucher specimen of the collected sample was deposited in the institutional herbarium and departmental museum for future reference.

Preliminary Pharmacognosy and extraction: The flowers of *A. speciosa* washed with distilled water to remove dirt and soil, and were shade dried. Routine pharmacognostic studies including organoleptic tests, macroscopic and microscopic observations were carried out to confirm the identity of the materials. The dried materials were powdered and passed through a 10-mesh sieve. The coarsely powdered material (500gm) was extracted thrice with ethanol (50% v/v). The extracts were filtered, pooled and concentrated at reduced temperature (-5 ^{0}C) on a rotary evaporator (Buchi, USA) and then freeze-dried (Freezone® 4.5, Labconco, USA) at high vacuum ($133X10^{-3}$ m Bar) and at temperature –40 ± 2 ^{0}C (yield 6.4%, w/w). Preliminary qualitative phytochemical screening of extract gives the positive test for alkaloids (Chao and Marderosian, 1973), lipids (Joshi and Garg, 1981), flavonoids, "steroids and triterpenoids", "coumarin and aryl esters" (Srivastava and Shukla, 1998), and proteins, fatty oils, resins and tannins. For the pharmacological tests the extract was suspended in double distilled water containing carboxy methyl cellulose (1% w/v, CMC).

The optimum condition for experiments were decided on the basis of initial pilot experiments performed on three rats per treatment. *A. speciosa* flower extracts were administered at up to $1 gkg^{-1}$ to individual rats in group. There was no mortality due to the treatment. Hence, further studies; 150 $mgkg^{-1}$ (p.o.) of maximum dose was employed.

The high performance thin layer chromatography (HPTLC) studies of the 50% ethanolic extract of *A. speciosa*, were carried out on pre coated silica gel plate (Merck 60 F 254) as the stationary phase and Toluene: Ethylacetate: Formic acid (5: 5: 1) as the mobile phase. The extract was spotted using a Camag Linomat IV spotter. These plates were observed at UV 254 and were scanned on TLC scanner III using CAT software.

Experimental animals: Sprague-Dawley rats weighing 140 - 160 g of either sex were purchased from the animal house of the Central Drug Research Institute, Lucknow. They were kept in departmental animal house in well cross - ventilated room at 27 ± 2 ^{0}C, and relative humidity

44 – 56%, light and dark cycles of 10 and 14 h, respectively, for one week before and during the experiments. Animals were provided with standard rodent pellet diet (Amrut, India) and the food was withdrawn 18 – 24 h before the experiment thought, water was allowed *ad libitum*. All the experiments were performed in the morning according to current guidelines for the care of the laboratory animals and the ethical guidelines for the investigation of experimental pain in conscious animals (Zimmerman, 1983). All the chemicals used were of the analytical grade from standard companies and the water represents the double distilled water. The standard orogastric cannula was used for oral drug administration.

Gastrointestinal motility: Animals were divided into four groups of six rats each and each animal was given orally 1 ml of charcoal meal (5% activated charcoal suspended in 1% CMC). Group I was administered with 1% CMC (10 $mlkg^{-1}$ p.o.) and animals in the groups II III and IV received extracted drug (50, 100 and 150 $mgkg^{-1}$, p.o.) immediately after the charcoal meal administration. The group V received atropine sulfate (0.1 $mgkg^{-1}$, i.p.) as standard drug. After 30 min animals were killed and the movement of charcoal from pylorus to caecum was measured. The charcoal movement in the intestine was expressed in terms of percentage (Lutterodt, 1989).

Castor oil-induced diarrhoea: The method of Awouters, *et al., (*1978) as modified by Nwodo and Alumanah (1991) was used. Briefly, rats fasted for 24h were randomly allocated to five groups of six animals each. Group I received 1% CMC (10 $mlkg^{-1}$, p.o.), groups II, III and IV received orally the drug extract (50, 100 and 150 $mgkg^{-1}$, respectively). The group V was given diphenoxylate HCl (5.0 $mgkg^{-1}$, p.o.) as suspension. After 60 min each animal was given with 2 ml of castor oil by orogastric cannula, and placed in a separate cage and observed for 4 h defecation. Transparent plastic dishes were placed beneath each cage and the characteristic diarrhoeal droppings were noted.

Castor oil-induced fluid accumulation: This was determined according to the method of Robert *et al.*, (1976) modified by Di Carlo *et al.*, (1994). The rats fasted for 24 h but allowed free access to water were randomised and allocated to five groups of six rats each. Group I (control) was administered 1% CMC (10 $mlkg^{-1}$, p.o.), group II was administered castor oil only (2 mL), groups III, IV and V were administered 50, 100 and 150 $mgkg^{-1}$ respectively, 1 h prior to castor oil administration to all the rats. After 30 min the rats were killed by cervical dislocation and exsanguinated; the small intestine was ligated both at pyloric sphincter and at the ileocaecal junctions. The entire small intestine was dissected out, its contents were expelled into a graduated measuring cylinder and the volume of the contents were recorded.

Statistical analysis: All the data were presented as mean ± standard error of the mean (mean ± SEM) and analysed by Wilcoxon Sum Rank Test (Padmanabha, *et al.*, 1982) followed by unpaired student"s t- test for the possible significant interrelation between the various groups. Significance of difference was accepted at $P < 0.05$.

Results

A. speciosa flowers were in sub – capitate cymes, peduncles 3 – 6 inch long, white tomentose out side, purple coloured. The corolla is long, tubular-infundibuliform, silky pubescent out side and glabrous inne, about 2.5 inch long. The plants microscopy and the macroscopy were matching completely with the previous descriptions found in old literatures (Anonymous, 1988; Bennet, 1976; Kapoor, 2000). The preliminary HPTLC studies revealed that the solvent system Toluene: Ethyl acetate: Formic acid (5: 5: 1) was ideal and gave the well- resolved peaks of the sample (Fig 1). The spots of the chromatogram were visualized at 366 nm with a 400 k filter at 0.52, 0.60, 0.70 and 0.77 Rf. The λ max for these major four spots were 332, 320, 288 and 291

respectively. The densitometric absorption at 200 nm was 31.29%, 27.79%, 22.06% and 14.89% respectively (Fig 2-5).

Gastrointestinal motility: As shown in Table 1 the charcoal meal travelled 72.77 % of the total length of the small intestine. The ethanolic extract of *A. speciosa* at the dose range of 50 – 150 $mgkg^{-1}$ caused a dose dependent decrease in the intestinal propulsion from 61.54% - 41.36% which is equivalent to 38.46% - 58.64% intestinal propulsive inhibition relative to control. The extract appears to be as inhibitory as atropine and reduce to a greater extent the intestinal propulsion ($P < 0.001$).

Castor oil-induced diarrhoea: Castor oil produced characteristic semisolid diarrhoea dropping in all the animals of the control group. The effect of *A. speciosa* flower extract at the dose levels of 100 and 150 $mgkg^{-1}$ caused a dose dependent decrease in the number of faecal matter passed by the rats. Diphenoxylate HCl, a standard antidiarrhoeal drug inhibited the diarrhoea by 63.23 % (Table 2).

Intestinal fluid accumulation: Treatment with *A. speciosa* extract (50 – 150 $mgkg^{-1}$) produced a significant ($P < 0.5 - P < 0.05$) and dose dependent reduction in the intestinal fluid accumulation from 9.97 % - 39.58 % (Table 3).

Discussion

In the present investigation, ethanolic extract of *A. speciosa* flower had shown dose dependent antidiarrhoeal activity in various validated models in rats. The activity was significant at a dose of 100 $mgkg^{-1}$ or more. The evidence presented here revealed that *A. speciosa* extract inhibits dose dependently the small intestinal propulsive movements in rats. The highest inhibition of gut motility was however, obtained with antimuscarinic drug atropine sulphate (Awouters, *et al.*, 1978). The ethanolic extract also showed a dose dependent decrease in castor oil induced diarrhoea like the standard antidiarrhoeal agent diphenoxylate HCl. Furthermore, this observation was also substantiated by significant action on castor oil induced intraluminal accumulation of fluid by indirect enteropooling assay in rats. These observations demonstrate the inhibitory effect of *A. speciosa* flower extract on castor oil induced diarrhoea, intraluminal fluid accumulation and peristaltic activity in small intestine. The action of castor oil as diarrhoea inductors has been largely studied and it is known, that its most active component is the ricinoleic acid, which produces an irritating activity of the small intestine. Prostaglandins contribute to the patho-physiological functions of the gastrointestinal tract and also act on the local electrical and mechanical activities of the ileal circular muscles (Sanders, 1984). Castor oil increases peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and waters (Bruton, 1985). The underlying mechanism may also be involved by which the plant extract produced relief in diarrhoea. Tannic acid and tannins are present in many plants and they denature proteins forming protein tannate, which makes the intestinal mucosa more resistant and reduces the secretion by virtue of which is said to be an excellent remedy for diarrhoea (Shobha and Thomas, 2001; Mukherjee, *et al.*, 1998). This is an augment with an findings showing that *Nelumbo nucifera* rhizome or plant constituents like bisnore (bisnordihydrotoxiferine) from *Strychnos trinervis* roots, tenatin, from flowers of *Egletes viscosai,* flavonoids like apigenin, flavone, kaempferol, morin and rutin using the presented experimental models in animals, while investigating their mechanism of action (De Melo, *et al.*, 1988; Di Carlo, *et al.*, 1993; Mukherjee, *et al.*, 1995; Rao, *et al.*, 1997). The more work is required for clear understanding of the mechanism of action. The plant has potent antidiarrhoeal activity, which justifies the ethnomedical claim.

Acknowledgements

The authors are thankful to Mr. Mithilesh Kumar Singh and Mr. Sudhir Kumar Singh for the technical help and Dr. R. L. S. Sikarwar, NBRI, Lucknow for taxonomical identification of the plant materials.

Table 1. Effect of *A. speciosa* extract on gastrointestinal transit in rats.

Treatment	Dose ($mgkg^{-1}$)	Mean intestinal length (cm)	Mean distance travelled by charcoal (cm)	Reduction (%)
Control (1%, 10 $mlkg^{-1}$ CMC)	---	79.3 ± 2.3	57.7 ± 4.1	27.23
A. speciosa	50	80.6 ± 5.3	49.6± 3.3	38.46
A. speciosa	100	87.3 ± 3.4	43.6 ± 2.4[a]	50.05
A. speciosa	150	83.9 ± 3.7	30.5 ± 3.1[b]	58.64
Atropine sulphate	0.1	85.7 ± 2.1	28.6 ± 1.6[b]	68.84

Values are mean ± SEM for six rats.
P: [a]<0.05 and [b]<0.001 compared to control

Table 2. Effect of *A. speciosa* extract on castor oil-induced diarrhoea in rats.

Treatment	Dose ($mgkg^{-1}$)	Total no of faecal matter	Reduction % (Inhibition)
Control (1%, 10 $mlkg^{-1}$ CMC)	---	68	----
A. speciosa	100	41	39.70
A. speciosa	150	30	55.88
Diphenoxylate HCl	5.0	25	63.23

Values presented are of six rats in each group.

Table 3. Effect of *A. speciosa* extract on castor oil-induced fluid accumulation in rats.

Treatment	Dose ($mgkg^{-1}$)	Intestinal fluid (ml)	% Inhibition
Control (1% CMC)	----	1.11 ± 0.06	-----
Castor oil	2 ml	3.41± 0.35[x]	-----
A. speciosa	50	2.97 ± 0.41	09.97
A. speciosa	100	2.41 ± 0.28[a]	29.32
A. speciosa	150	2.06 ± 0.17[a]	39.58

Values are mean ± SEM for six rats.
P: [x]< 0.001 compared to control.
P: [a]< 0.05 compared to castrol oil group.

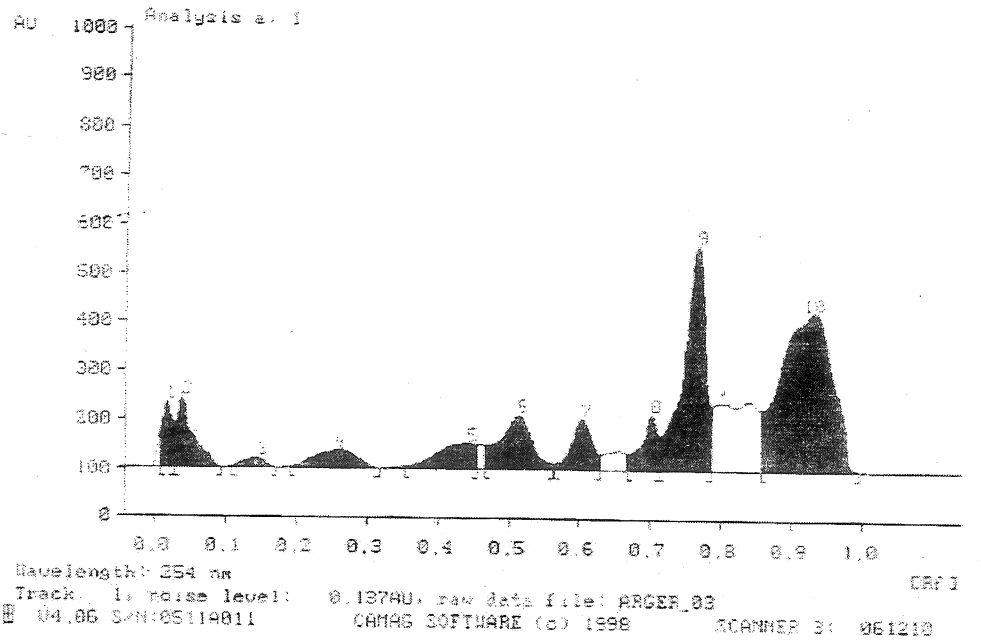

Fig.1.HPTLC densitometric scan of 50% ethanolic flower extract of *Argyreia speciosa* Sweet.

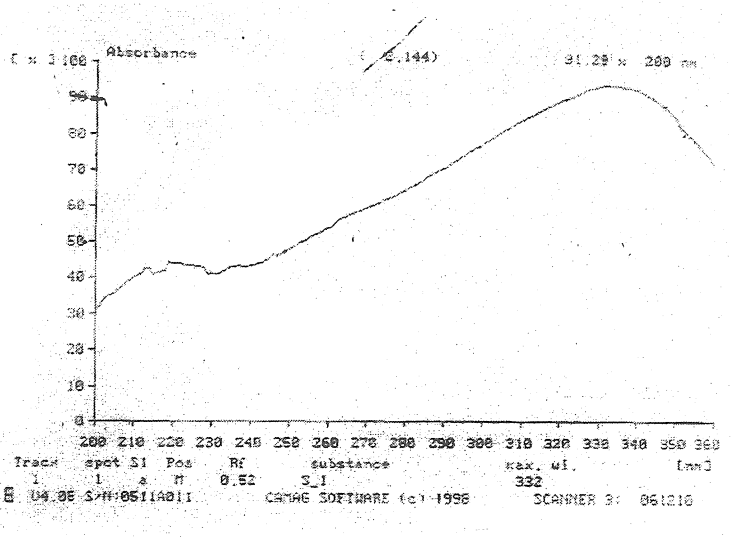

Fig.2. The λ max of one of the major spots of 50% ethanolic flower extract of *Argyreia speciosa* Sweet is 332 and densitometric absorption was 31.29% at 200nm.

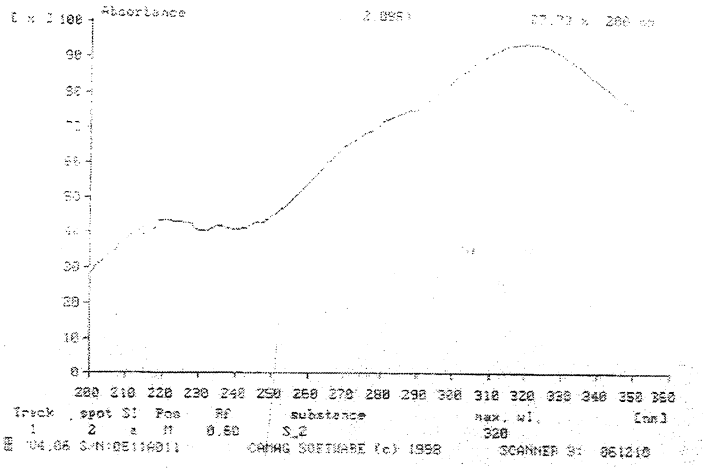

Fig.3. The λ max of one of the major spots of 50% ethanolic flower extract of *Argyreia speciosa* Sweet is 320 and densitometric absorption was 27.79% at 200nm.

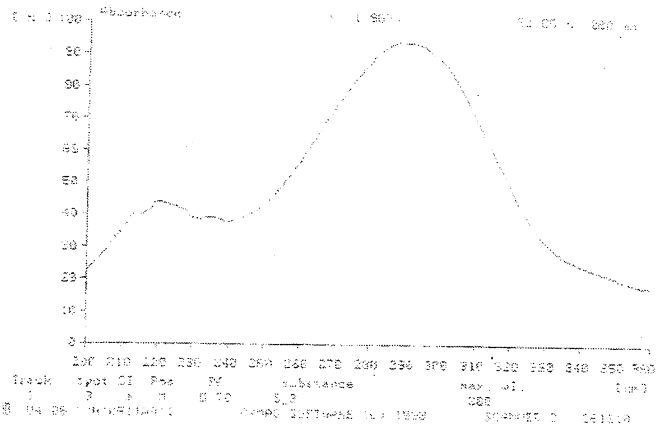

Fig.4. The λ max of one of the major spots of 50% ethanolic flower extract of *Argyreia speciosa* Sweet is 288 and densitometric absorption was 22.06% at 200nm.

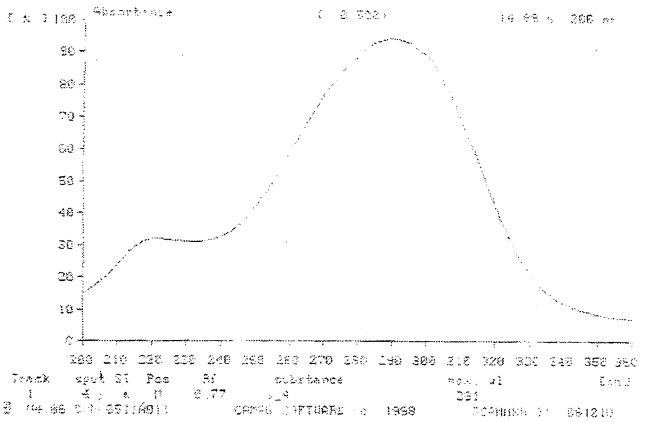

Fig.5. The λ max of one of the major spots of 50% ethanolic flower extract of *Argyreia speciosa* Sweet is 291 and densitometric absorption was 14.89% at 200nm.

References

Anonymous, (1979). Diarrhoeal Disease Control Programme: Weekly Epidemic Record. Vol.16, 121.

Anonymous, (1988). The Wealth of India: Raw materials, PID, CSIR, New Delhi, pp 418 – 419.

Awouters, F., Niemegeers, C. J. E., Lenaerts, F. M., Janssen, P. A. J. (1978). Delay of castor oil induced diarrhoea in rats; a new way to evaluate the prostaglandin synthesis. *J. Pharm. Pharmacol.* 30: 41 – 45.

Bennet, S. S. R. (1976). Flora of Howrah District. Int. Book Distributors, Dehradun.

Bhargava, N. C., Singh, O. P. (1978). Fortege: An indigenous drug in common sexual disorders in males. *Mediscipe*. 21: 140 – 144.

Black, R. E., Brown, K. H., Becker, S., Yunus, M. (1982). Longitudinal studies of infectious diseases and physical growth of children in rural area of Bangladesh. *American Epidemiol.* 115: 305 –314.

Bruton, L. L. (1985). Agent affecting gastrointestinal water flux and motility, digestants; and bile acids, In : Gillman, A. G., Rall, T. W., Nies, A. S., Taylor, P., (Eds.), The Pharmacological Basis of Therapeutics. Vol. 2, 8th ed. Mc Graw – hill International Editions. New York, pp 914.

Chao, J. M., Marderosian, A. H. (1973). Identification of ergoline alkaloids in the genus *Argyreia* and related genera and there chemo taxonomic implications in the Convolvulaceae. *Phytochem.* 12: 2435 – 2440.

Chopra, R. N., Nayar, S. L., Chopra, I. C. (1956). Glossary of Indian Medicinal Plants. CSIR, New Delhi, 24.

De Melo, M. F. F., Thomas, G., Mukherjee, R. (1988). Antidiarrhoeal activity of bisnordihydrotoxiderine isolated from the root bark of the *Strychnos trinervis* (Vell.) Mart. (Loganiaceae). *J. Pharm. Pharmacol.* 49: 79 – 81.

Desai, H. K., Gawad, D. H., Govindachari, T. R., Joshi, B. S., Kamat, V. N., Parthasarathy, P. C., Ramachandra, K. S., Shanbhag, M, N., Sidhaye, A. R., Vishwanathan, N. (1975). Chemical investigation of some Indian plants, VIII, *Indian J Chem.* 13: 97 - 98.

Di Carlo, G., Gautore, A., Izzo A., Maiolino, P., Mascolo, N., Viola, P., Diurno, M. V., Capasso, F. (1993). Inhibition of intestinal motility and secretion by flavonoids, in mice and rats; structure-activity relationships. *J. Pharm. Pharmacol.* 45:1054 – 1058.

Di Carlo, G., Mascolo, N., Izzo, A. Capasso, F., Autore, G. (1994). Effect of Quercetin on gastrointestinal tract in rats and mice. *Phytotherapy Research.* 8, 42 – 45.

Duthie, J. F. (1960). Flora of The Upper Gastric Plain, Botanical Survey of India, Govt. of India, vol. 1, p. 561.

Jain, S. K., Tarafder, C. R. (1970). Medicinal Plantlore of The Santals, A revival of P. O. Bodding's work. *Econ. Bot.* 24: 241 – 278.

Joshi, R., Garg, B. D. (1981). Analysis of the fatty acid composition of *Argyeria speciosa, J. Sci. Res.* 3: 195 – 198.

Kapoor, L. D. (2000). C. R. C. Hand Book of Ayurvedic Medicinal Plants, Publication: herbal references library. pp. 48 – 49.

Lutterodt, G. D. (1989). Inhibition of gastrointestinal release of acetylocholine by quercetine as a possible mode of action of *Psidium guajava* leaf extracts in the treatment of acute diarrhoeal disease. *J. Ethnopharmacol.* 25: 235 – 247.

Mishra, S. H., Chaturvedi, S. C. (1978). Antibacterial and antifungal activity of the oil and unsaponifiable matter of *Argyreia speciousa* Sweet. *Indian Drugs and Pharm. Ind.* 13: 29 –31.

Mukherjee, P. K., Das, J., Balasubramanian, R., Saha, K., Pal, M., Saha, B. P. (1998). Antidiarrhoeal evaluation of *Nelumbo nuciferai* rhizome extract. *Indian J. Pharmacol.* 22: 262-265.
Mukherjee, P. K., Saha, K., Murugesan, T., Mandal, S. C., Pal, M., Saha, B. P. (1998). Screening of antidiarrhoeal profile of some medicinal plant extract of a specific region of West Bangal, India. *J. Ethnopharmacol.* 60: 85 – 89.

Nwodo, O. F. C., Alumanah, E. O. (1991). Studies on *Abrus precatorious* seed II: Antidiarrhoeal activity. *J. Ethnopharmacol.* 31: 395 – 398.

Padmanabha, P.N., Ramaswamy, S., Gopalakrishinan, V., Ghosh, M.N. (1982). Effect of cholinergic drugs on acute and chronic morphine dependence. *Arch. Int. Pharmacodyn.* 257: 147-154.

Rao, Ch. V., Ojha, S K., Amresh., Mehrotra, S and Pushpangadan, P. (2003) Analgesic, anti-inflammatory and antiulcerogenic activity of the unripe fruits of *Aegle marmelos. Acta Pharmaceutica Turcica.* 45: 85 – 91.

Rao, Ch. V., Amresh., Reddy, G. D., and Shirwaikar, A. (2004). Ethnopharmacological value of *Cissampelos pareira* extract in experimentally induced diarrhoea. *Acta Pharmaceutica* 54:27 – 35.

Rao, V. S. N., Santos, F. A., Sobreira, T. T., Souza, M. F., Melo, C. L., Silveira, E. R. (1997). Investigation of the gastroprotective and antidiarrhoeal properties of ternatin, a tetramethoxyflavone from *Egletes viscose. Planta Medica.* 63: 146 – 151.

Robert, A., Nezamis, J. E., Lancaster, C., Hanchar, A. I., Klepper, M. S. (1976). Enteropooling assay: A test for diarrhoea produced by prostaglandins. *Prostaglandins.* 11, 809 – 814.

Sanders, K. M. (1984). Evidence that prostaglandins are local regulatory agents in canine ileal circular muscles. *American J. Physiol.* 246: G361- G366.

Shobha, F. G., Thomas, M. (2001). Study of antidiarrhoeal activity of four medicinal plants in castor oil induced diarrhoea. *J. Ethnopharmacol.* 76: 73 – 76.
Srivastava, A., Shukla, Y. N. (1998). Aryl esters and a coumarin from *Argyeria speciosa. Indian J. Chem.* 37 B: 192 – 194.

Syder, J. D., Merson, M. H. (1982). The magnitude of global programme of acute diarrhoeal disease: A review of active surveillance data. *Bulletin of World Health Organisation.* 60, 605 – 613.

Zimmerman, M. (1983). Ethical guidelines for investigation of experimental pain in conscious animal. *Pain.* 16: 109 – 110.

Received: 10.05.2004
Accepted: 24.06.2004