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Research Article

ASSESSMENT OF ANALGESIC, ANTIDIARRHOEAL AND CYTOTOXIC ACTIVITY OF ETHANOLIC EXTRACT OF THE WHOLE PLANT OF *BACOPA MONNIERI* LINN

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ABSTRACT

The ethanol extract of the whole plant of *Bacopa monnieri* Linn. (Scrophulariaceae) was investigated for its possible analgesic, antidiarrhoeal and cytotoxic activities in animal models. The extract produced significant writhing inhibition in acetic acid-induced writhing in mice at the oral dose of 250 mg/kg & 500 mg/kg body weight ($p<0.001$) comparable to the standard drug Diclofenac sodium at the dose of 25 mg/kg of body weight. The extract showed antidiarrhoeal activity on castor oil induced diarrhoea in mice; it increased mean latent period and decreased the frequency of defecation significantly at the oral dose of 500 mg/kg body weight comparable to the standard drug Loperamide at the dose of 50 mg/kg of body weight. The crude extract produced the most prominent cytotoxic activity against brine shrimp *Artemia salina* (LC_{50} = 26.30 µg/ml).

Key words: *Bacopa monnieri,* analgesic, antidiarrhoeal, cytotoxic, A*rtemia salina*

INTRODUCTION

Bacopa monnieri Linn. also referred to as water hyssop, thyme-leaved graticula in English, and "Brahmi" in Bangla, has been used in the Ayurvedic system of medicine for centuries. *Bacopa monnieri*, a member of the Scrophulariaceae family, is a small, creeping herb with numerous branches, small oblong leaves, and light purple flowers. In India and the tropics it grows naturally in wet soil, shallow water, and marshes. The herb can be found at elevations from sea level to altitudes of 4,400 feet, and is easily cultivated if adequate water is available. Flowers and fruit appear in summer and the entire plant is used medicinally[1,2]. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration[3] and to provide relief to patients with anxiety or epileptic disorders[1]. The plant has also been used in India and Pakistan as a cardiac tonic, digestive aid, and to improve respiratory function in cases of bronchoconstriction[4]. The plant is used against fever, arthritis, rheumatism, diabetes, cough, bed ulcers and tumors, diarrhea and bronchitis. The plant forms an important ingredient of Ayurvedic preparations such as Brahmigrihitam, Brahmirasayanm, etc[5]. Recent research has focused primarily on Bacopa's cognitive-enhancing effects, specifically memory, learning, and concentration and results support the traditional Ayurvedic claims. Research on anxiety, epilepsy, bronchitis and asthma, irritable bowel syndrome, and gastric ulcers also supports the Ayurvedic uses of Bacopa. Bacopa's antioxidant properties may offer protection from free radical damage in cardiovascular disease and certain types of cancer.

In previous studies, *Bacopa monnieri* was reported as hypotensive and vasodilator by Kamkaew et al[6], Carlo et al reported the Effects on cognitive performance, anxiety, and depression in the Elderly[7], Cognitive enhancement and neuroprotective effects in Alzheimer's disease model was reported by Uabundit et al[8], Jadiya reported the anti-Parkinsonian effects of the plant extract[9], Steven et al reported its Chronic Effects on Human Memory[10], Watoo et al reported the Determination of Saponin Glycosides in the plant by Reversed Phase High Performance Liquid Chromatography[11], Chillara et al reported the Triterpenoid glycosides from Bacopa monnieri [12], the plant was reported to increases cerebral blood flow in rat independent of blood pressure by Natakorn et al[13].

Since no literature is currently available to substantiate analgesic, antidiarrhoeal and cytotoxic activities from ethanol extract of *Bacopa monnieri*, therefore the present study is a part of our on-going pharmacological screening of selected Bangladeshi medicinal plants[14-18] and designed to provide scientific evidence for its use as a traditional folk remedy by investigating the analgesic, antidiarrhoeal and cytotoxic activities that also confirm its use as pain killer and other pathological conditions where antidiarrhoeal and cytotoxic medications are implicated.

MATERIALS AND METHODS

Plant material collection and extraction

For this present investigation, *Bacopa monnieri* Linn. (Scrophulariaceae) was collected from Khulna University campus, Khulna, Bangladesh, 20 June 2009 at morning time and identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession No: 34405) and a voucher specimen also deposited there. About 400 gm of powdered leaves were taken in a clean, flat-bottomed glass container and soaked in 1,300 ml of 80% methanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of cotton followed by a filtration through Whatmann filter paper and the filtrate thus obtained was concentrated using a rotary evaporator (Bibby RE200, Sterilin Ltd., U.K.) to get the crude extract.

Drugs

Diclofenac Sodium (Opsonin Pharmaceuticals Ltd., Bangladesh), Loperamide (Square Pharmaceuticals Ltd., Bangladesh).

Animals

Young Swiss-albino mice of either sex, weighing 20-25 gm, purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) were used for the test. The animals were kept at animal house (Pharmacy Discipline, Khulna University) for adaptation after their purchase under standard laboratory conditions (relative humidity 55 - 65%, room temperature 25.0 ± 2.0°C and 12 h light-dark cycle) and fed

with standard diets (ICDDR, B formulated) and had free access to tap water. The experimental met the national guidelines on the proper care and use of animals.

PHARMACOLOGICAL STUDIES

Analgesic activity

Analgesic activity of the crude extract was tested using the model of acetic acid-induced writhing in mice[19,20]. The experimental animals were randomly divided into four groups, each consisting of ten animals. Group I was treated as 'control group' which received 1% (v/v) Tween-80 in water at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug Diclofenac sodium at dose of 25 mg/kg of body weight; group III and group IV were test groups and were treated with the extracts at dose of 500 mg/kg of body weight respectively. Control vehicle, standard drug and extracts were administered orally, 30 minutes prior to acetic acid (0.7%) injection. Then after an interval of 15 minutes, the number of writhes (squirms) was counted for 5 min.

Antidiarrhoeal activity

Antidiarrhoeal activity of the ethanol extract of whole plant of *Bacopa monnieri* was tested using the model of castor oil-induced diarrhoea in mice[21]. The mice were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhoea were selected for the final experiment. The test animals were randomly chosen and divided into three groups having five mice in each. Group-I was kept as control and received 1% Tween-80 at the dose of 10 ml/kg of body weight; group II was treated as 'positive control' and was given the standard drug Loperamide at a dose of 50 mg/kg of body weight; group III was test group and was treated with the extract at a dose of 500 mg/kg of body weight. Control vehicle, standard drug and the extract were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hours study after the castor oil administration. Number of stools or any fluid material that stained the adsorbent paper was counted at each successive hour during the experiment. The latent period of each mouse was also counted. At the beginning of each hour old papers were replaced by the new ones.

Cytotoxicity test

The method of Meyer *et al* [22] with some modifications was adapted to study the general toxicity of *Bacopa monnieri* Linn. The brine shrimp eggs were hatched in a conical flask containing brine shrimp medium (300 ml). The flasks were well aerated with the aid of an air pump, and kept in a water bath at 29 – 30° C. A bright light was left on. The nauplii hatched within 48 hour. The extract was dissolved in brine shrimp medium with addition of few drops of 5% DMSO to obtain a concentration of 5, 10, 20, 40, 80 and 160 µg/ml. Each preparation was dispensed into clean test tubes in 10 ml volumes and tested in duplicates. For control, same procedure was followed except test samples. A series of the same concentration as of the sample was prepared for positive control, chloramphenicol[23]. After marking the test tubes properly, 10 living shrimps were added to each of the test tubes with the help of a Pasteur pipette. The test tubes containing the sample, control and positive control were then incubated at 29°C for 24 hour in a water bath, after which each tube was examined and the surviving brine shrimps counted and recorded. From this, the percentage of mortality was calculated at each concentration. The LC_{50} values were calculated with best fit line by using Microsoft Excel 2007.

Table 1: Result of the Analgesic effect of Ethanolic extract of whole plant of *Bacopa monnieri* Linn.

Animal group/ Treatment	Number of writhes (% writhing)	Inhibition (%)
Control 1% tween-80 in water, p.o.	15±0.835 (100)	-
Positive control Diclofenac sodium 25 mg/kg, p.o.	4.4±0.51* (29.33)	70.67
Test group - 1 Ethanolic extract 250 mg/kg, p.o.	9.6±2.25** (64)	36
Test group - 1 Ethanolic extract 250 mg/kg, p.o	5.8±0.75* (38.67)	61.33

Values are expressed as Mean S.E.M (n=5), *P<0.001, **P<0.01,
% = Percentage, p.o. = per oral.

Table 2: Effect of *Bacopa monnieri* Linn. on the latent period of Castor oil induced diarrheal episode in mice

Animal Group/ Treatment	Dose (/kg.p.o)	latent period(hr)
Control (1% tween-80)	10 ml	0.75±0.06
Positive control Loperamide	50 mg	2.28±0.20*
Test group EtOH. Extract	250 mg	1.24±0.18**
Test group EtOH. Extract	500 mg	1.61±0.16***

Values are expressed as Mean S.E.M (n=5), *P<0.001, **P<0.05,
***P<0.01, % = Percentage, p.o. = per oral, EtOH = Ethanol

Table 3: Effect of *Bacopa monnieri* Linn. on the basis of mean of feces of castor oil induced diarrheal episode in mice

Animal Group/ Treatment	Dose (/kg.p.o)	Mean of feces
Control (1% tween-80)	10 ml	7.6±1.43
Positive control Loperamide	50 mg	2.4±0.51*
Test group EtOH. Extract	250 mg	5.4±0.93*
Test group EtOH. Extract	500 mg	3.6±0.98*

Values are expressed as Mean S.E.M (n=5), *P<0.01,
% = Percentage, p.o. = per oral, EtOH = Ethanol

Table 4: Result of brine shrimp lethality bioassay of Ethanolic extract of whole plant of *Bacopa monnieri* Linn.

Test sample	Conc. (µg/ml)	Log (Concentration)	No. of alive shrimp	% mortality	LC_{50} (µg/ml)	LC_{90} (µg/ml)
Ethanolic extract of *Bacopa monnieri*.	5	0.69	10	0	26.30	141.25
	10	1	6.5	35		
	20	1.3	6.0	40		
	40	1.6	3.5	65		
	80	1.9	3.0	70		
	160	2.2	0.5	95		
	320	2.5	0	100		

Conc. = Concentration, % = Percentage, No. = Number, LC_{50} = 50% lethal concentration, LC_{90} = 90% lethal concentration.

RESULTS

Analgesic activity

The analgesic effect of the ethanolic extract of whole plant of *Bacopa monnieri* was tested on acetic acid-induced writhing in mice. At dose of 250 mg/kg & 500 mg/kg of body weight, the extract produced about 36% & 61.33% writhing inhibition in test animals (Table 1). The results were statistically significant ($P < 0.1$ & $P < 0.001$) and were comparable to the standard drug Diclofenac sodium, which showed about 70.67% writhing inhibition at the dose of 25 mg/kg ($P < 0.001$).

Antidiarrhoeal activity

Antidiarrhoeal activity of the ethanolic extract of whole plant of *Bacopa monnieri* was tested by castor oil-induced diarrhoea in mice. Diarrhoeal initiation time and the number of stools excreted by the animals in 4 hours were collected. The extract caused an increase in latent period 1.24 and 1.61 hour i.e. delayed the onset of diarrhoeal episode of 250 & 500 mg/kg body of weight significantly which was comparable to the standard drug Loperamide at the dose of 50 mg/kg body weight in which the resulted value was 1.61hour ($P<0.01$) (Table 2). The selected concentration of the extract also showed a decrease mean of feces with 5.4 and 3.6 at a dose of 250 & 500 mg/kg body of weight whereas Loperamide, standard antidiarrhoeal agent showed 3.6 mean no. of feces (Table 3).

Cytotoxic activity

In brine shrimp lethality bioassay (Table 4), the extract showed lethality against the brine shrimp nauplii. It showed different mortality rate at different concentrations. From the graph the concentrations at which 50% mortality (LC_{50}) & 90% mortality (LC_{90}) of brine shrimp nauplii occurred were obtained by extrapolation & the values were found to be 26.30µg/ml & 141.25µg/ml respectively for the crude extract of the of *Bacopa monnieri* Linn. From the graph the concentrations at which 50% mortality (LC_{50}) & 90% mortality (LC_{90}) of brine shrimp nauplii occurred were obtained by extrapolation & the values were found to be 18.62µg/ml & 134.9µg/ml respectively for the Chloramphenicol.

DISCUSSION

Plants are employed as important source of medication in many traditional medications[24, 25, 26]. *Bacopa monnieri* contain steroidal saponins, alkaloids, d-mannitol, stigmasterol, beta-sitosterol, luteolin, glucoside, brahmine, bacoside A and B, herpestine, luteolin [27]. Preliminary phytochemical screening of the extract showed the presence of reducing sugars, alkaloids, glycosides, tannins, saponins, gums, and steroids. Polyphenolic compound like tannins have been reported to have multiple pharmacological effects, including analgesic and antidiarrhoeal activities. Roome *et al.*[28] (2008), showed that plant contains Pentacyclic triterpenes may caused the inhibition pain mice. This study also revealed that the presence of Benzoquinones also can inhibit the Lipooxygenase pathways which support the uses of *Bacopa monnieri* in folk medicine against diarrhoea. Presence of saponins and tannins also involved in the antidiarrhoeal activities. Another study conducted by Ahmed *et al.*[29] (2007) showed the presence of steroids, alkaloids and glycosides can caused the analgesic and antidiarrhoeal activities.

Analgesic activity of the ethanol extract of whole plant of *Bacopa monnieri* was tested by acetic acid-induced writhing model in mice. Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings[30]. Increased levels of PGE2 and PGF2α in the peritoneal fluid have been reported to be responsible for pain sensation caused by intraperitoneal administration of acetic acid[31]. The extract produced significant writhing inhibition comparable to the standard drug Diclofenac sodium. The polar compounds present in the plant extract may be responsible for the obtained analgesic activity. Based on this result it can be concluded that the ethanol extract of *Bacopa monnieri* possess analgesic activity.

Antidiarrhoeal activity of the ethanol extract of *Bacopa monnieri* was tested by using the model of castor oil-induced diarrhoea in mice[32]. Number of mechanisms have been previously proposed to explain the diarrhoeal effect of castor oil including inhibition of intestinal Na+, K+- ATPase activity to reduce normal fluid absorption[33] activation of adenylate cyclase or mucosal cAMP mediated active secretion[34], stimulation of prostaglandin formation[35], platelet activating factor and recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil[36.] However, castor oil induced diarrhoea when it mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinolic acid remains in the intestine and produces its absorptive or secretory effect. The ricinolic acid thus liberated readily forms of ricinoleate salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. Generally ricinoleate salts stimulates the intestinal epithelial cells adenyl cyclase[37] or released prostaglandin[38]. The extract caused and increased in latent period and decreased the frequency of defecation as well as the number of total stool count. Generally the ethanol extract of whole plant of *Bacopa monnieri* experimentally inhibited the castor oil-induced diarrhoea.

The cytotoxic activity of the ethanol extract of *Bacopa monnieri* was tested by using brine shrimp lethality bioassay. It is a recent development in the bioassay for the bioactive compounds. The plant is reported to contain Saponins[4]. There is growing interest in natural saponins caused as much by the

scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of pharmacological activities; for instance, bactericidal, antiviral, cytotoxic, analgesic, anti-inflammatory, anti-cancer and antiallergic[39,40]. Brine shrimp lethality bioassay indicates cytotoxicity as well as a wide range of pharmacological activities such as antimicrobial, pesticidal, antitumor, etc[41]. The extract was found to show potent activity against the brine shrimp nauplii. Therefore the positive response obtained in this assay suggests that the extract may contain antitumor, antibacterial or pesticidal compounds which support the use of the plant in traditional medicine.

REFERENCES

1. Chopra RN. Indigenous Drugs of India. 2nd ed. Calcutta, India: U.N. Dhur and Sons; 1958:341.
2. Bone K. Clinical Applications of Ayurvedic and Chinese Herbs: Monographs for the Western Herbal Practitioner. Warwick, Queensland: Phytotherapy Press; 1996.
3. Mukherjee DG, Dey CD. Clinical trial on Brahmi. I. J Exper Med Sci 1966;10:5-11.
4. Nadkarni KM. The Indian Materia Medica. Columbia, MO: South Asia Books; 1988:624-625.
5. Joshi .S G, Medicinal Plants, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India, 2000, 363.
6. Kamkaew N, Scholfield CN, Ingkaninan K, Maneesai P, Parkington HC, Tare M, Chootip K, Bacopa monnieri and its constituents is hypotensive in anaesthetized rats and vasodilator in various artery types. J Ethnopharmacol., 2011, 1;137(1):790-5
7. Carlo Calabrese, William L. Gregory, Michael Leo, Dale Kraemer, Kerry Bone, Barry Oken, Effects of a Standardized *Bacopa monnieri* Extract on Cognitive Performance, Anxiety, and Depression in the Elderly: A Randomized, Double-Blind, Placebo-Controlled Trial, J Altern Complement Med. 2008, 14(6): 707–713.
8. Uabundit N, Wattanathorn J, Mucimapura S, Ingkaninan K, Cognitive enhancement and neuroprotective effects of Bacopa monnieri in Alzheimer's disease model, J Ethnopharmacol., 2010, 8;127(1):26-31.
9. Jadiya P, Khan A, Sammi SR, Kaur S, Mir SS, Nazir A., Anti-Parkinsonian effects of Bacopa monnieri: insights from transgenic and pharmacological Caenorhabditis elegans models of Parkinson's disease. Biochem Biophys Res Commun. 2011, 7;413(4):605-10.
10. Steven Roodenrys, Dianne Booth, Sonia Bulzomi, Andrew Phipps, Caroline Micallef, Jaclyn Smoker, Chronic Effects of Brahmi (Bacopa monnieri) on Human Memory, Neuropsychopharmacology, 2002, 27: 2.
11. Watoo Phrompittayarat, Sakchai Wittaya-areekul, Kanchalee Jetiyanon, Waraporn Putalun, Hiroyuki Tanaka, Kornkanok Ingkaninan, Determination of Saponin Glycosides in Bacopa monnieri by Reversed Phase High Performance Liquid Chromatography, Thai Pharm Health Sci J, 2007, 2(1):26-32.
12. Chillara Sivaramakrishna, Chirravuri V Rao, Golakoti Trimurtulu, Mulabagal Vanisree, Gottumukkala V Subbaraju, Triterpenoid glycosides from Bacopa monnieri, Phytochemistry, 2005, 66: 23, 2719-2728.
13. Kamkaew, N., Norman Scholfield, C., Ingkaninan, K., Taepavarapruk, N. and Chootip, K. *Bacopa monnieri* Increases Cerebral Blood Flow in Rat Independent of Blood Pressure. Phytother. Res, 2012, 3.
14. Naheed M, Akter KA, Tabassum F, Mawla R, Rahman M, Factors contributing the outcome of Schizophrenia in developing and developed countries: A brief review., 2012, Int. Current. Pharm. J. 1(4):81–85
15. Nayeem AA, Khatun A, Rahman MS, Rahman M., Evaluation of phytochemical and pharmacological properties of Mikania cordata (Asteraceae) leaves. J. of Pharmacognosy and Phytotherapy, 2011; 3(8): 111-123.
16. Rahman M, Khatun A, Rahman SM, Rashid MA Antioxidant, Antimicrobial and Cytotoxic activities of Vitis trifolia Linn., 2010, J. of Dhaka Int. Univ. 2010; 1(1): 181-184.
17. Ahmed F, Ohtsuki T, Rahman M, Sadhu SK, Toume Kazufumi, Ishibashi M., Cryptolepine, isolated from Sida acuta, sensitizes human gastric adenocarcinoma cells to TRAIL-induced apoptosis. Phytotherapy Research, 2010, 25(1):147–150
18. Bokshi B, Siraj MA, Ahmed M.I, Karmakar UK, Sadhu SK., Assessment of Antimicrobial and Cytotoxic Activities of Ethanolic Extract of Leaves of acalypha hispida, International Journal on Pharmaceutical Sciences and Research, 2012, 3(6): 1705-1708.
19. Khan MA, Kust GS, Barth H-J & Boer B. Sabkha Ecosystems. 2006; Vol:*II*: pp 129-153.
20. Evans WC. Trease and Evan's Textbook of Pharmacognosy. 1989 13th ed, Cambidge University Press, London,pp 546.
21. Ahmed F., Selim MST., Das AK, Choudhuri MSK. Anti-inflammatory and antinociceptive activities of *Lipia nodiflora* Linn. Pharmazie 2004;59:329-330.
22. Whittle BA. The use of changes in capillary permeability in mice to distinguish between narcotic and non-narcotic analgesics. Br. J. Pharmacol. Chemother.1964; 22: 246-253.
23. Meyer BN, Ferrigni NR, Putnam JB, Jacobsen LB, Nichols DE, McLaughlin JL. Brine shrimp: a convenient general bioassay for the active plant constituents. Planta Med. 1982; 45:31-34.
24. Grover JK, S Yadav, V Vats. Medicinal plants of India with anti-diabetic potential. J. Ethnopharmacol. 2002; 81(1): 81-100.
25. Keung WM, BL Vallee. Kudzu root: An ancient chinese source of modern antidipsotropic agents. Phytochemistry. 1998; 47 (4): 499-506.
26. Neves JM, C Matos, C Moutinho, G Queiroz, LR Gomes. Ethnopharmacological notes about ancient uses of medicinal plants in Trás-os-Montes (northern of Portugal). J. Ethnopharmacol. 2009; 124(2): 270-283.
27. Ghani A. Medicinal Plants of Bangladesh-Chemical constituents and uses, 2nd edition, The Asiatic Society of Bangladesh, Dhaka. 2003; pp.323-5,505-7.
28. Roome T, Dar A, Naqvi S, Ali S, Choudhary MI. A study on antioxidant, free radical scavenging, anti-inflammatory and hepatoprotective actions of *Aegiceras corniculatum* (stem) extracts. J Ethnopharmacol. 2008; 118: 514-521.
29. Ahmed F, Al Mamun AH, Shahid IZ, Rahman AA, Sadhu SK. Antinociceptive, antidiarrhoeal and cytotoxic activity of *Aegiceras corniculatum.* Orient Pharm Exp Med. 2007; 7(2):191-196.
30. Taesotikul T, Panthong A, Kanjanapothi D, Verpoorte R, Scheffer JJC. Anti-inflammatory, antipyretic and antinociceptive activities of *Tabernaemontana pandacaqui* Poir. J. Ethnopharmacol. 2003; 84: 31-33.
31. Derardt, R, Jougney S, Delevalcee F, Falhout M. Release of prostaglandins E and F in an algogenic reaction and its inhibition. Eur. J. Pharmacol. 1980; 51: 17-24.
32. Chatterjee TK, (1993) "Handbook *of laboratory Mice and Rats*" First Edition, Jadavpur University, India, pp. 133-139.
33. Ganinella TS, P Bas. Laxatives: an update on mechanism of action. Life Sci. 1978;23:1001-10.
34. Capasso F, N Mascolo, AA Izzo, TS Ganginella. Dissociation of castor oil-induced diarrhoea and intestinal mucosal injury in rat: effect of NG-nitro-L-arginine methyl ester. British J. Pharmacol. 1994; 113: 1127-30.
35. Galvez A, ME Zarzuelo, MD Crespo, M Lorente, A Ocete, J Jimenez. Anti-diarrhoeal activity of *Euphorbia hirta* extract and isolation of an active flavonoidal constituent. Planta Med.1993; 59:333-6.
36. Mascolo N, AA Izzo, G Autore, F Barbato, F Capasso. Nitric oxide and castor oil-induced diarrhoea. J. Pharmacol. Exp. Ther. 1994; 268:291-5.
37. Racusen LC, H.J. Binder. Ricinolic acid stimulation of active anion secretion in colonic mucosa of the rat. J. Clin. Invest. 1979; 63: 743-749.
38. Beubler E, H. Juan. Effect of Ricinolic acid other Laxatives in Net Water Flux and Prostaglandin E release by the Rat colon. J. Pharm. Pharmacol. 1979;31: 681-685.
39. Ksouri R, Falleh H, Megdiche W, Trabelsi N, Mhamdi B, Chaieb K, Bakrouf A, Magne C, Abdelly C. Antioxidant and antimicrobial acivities of the edible medicinal halophyte Tamarix gallica L. and related polyphenolic constituents. Food Chem Toxicol. 2009;47:2083-2091.
40. Balasundaram N, Sundaram K, Sammar S. Phenolic compounds in plants and agri-industrial by-products. Antioxidant activity, occurance and potential uses. Food Chem. 2006;1:191-203.
41. Anderson JE, Chang CJ, McLaughlin JL. Bioactive components of *Allamanda nodiflora* Linn. J.Nat. Prod. 1988; 51: 307-308.

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