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EVALUATION OF ANALGESIC ACTIVITY OF VARIOUS EXTRACTS OF *SIDA TIAGII* BHANDARI

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Abstract: *Sida tiagii* Bhandari mostly found in India and Pakistan which belongs to family Malvaceae, is traditionally used as analgesic, anti-inflammatory, sedative, anxiolytic, anti-seizure and anti-platelet. The present study was done to explore the analgesic activity of various extracts of fruits of the plant *Sida tiagii* Bhandari. The grinded fruits were extracted with 90% ethanol and partitioned with n-hexane (n-hexane extract; HS) and ethyl acetate (ethyl acetate extract; EAS), successively. The residual ethanol fraction (residual ethanol extract; RES) was also prepared by drying on water bath separately. All three extracts were administered orally at a dose of 200 mg/kg and 500 mg/kg of body weight. The analgesic activity of above extracts was evaluated by using acetic acid induced writhing, tail immersion and tail flick tests in Swiss albino mice. The EAS extract was found to reduce pain and RES extract of *Sida tiagii* B. was found to have good analgesic activity in comparison to other extracts.

Keywords: *Sida tiagii* B., acetic acid, analgesic, tail flick test, writhing test

Algesia or pain is a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. The ascending pathway of pain includes the contralateral spinothalamic tract, lateral pons, mid brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain. Many drugs used to relieve the pain and a few drugs like morphine and aspirin have been extensively used for the last three decades. Most of the pain-relieving chemicals produced pronounced side-effects on the physiology of the body. In the indigenous system of medicine, several plants possess an analgesic property and many investigators screened the plant crude extracts for their analgesic property (1).

Sida tiagii Bhandari (*Sida pakistanica* B.; family Malvaceae), a native species of the India and Pakistan desert area, popularly known as "Kharinti" in India; is used in the folk medicine as blood purifier, tonic and muscle strengthener (2). The reported activities of *Sida tiagii* include anxiolytic, antiseizure (3), spasmogenic, spasmolytic (4) and antiplatelets (5). The other species from Sida genus like *Sida cordifolia, Sida acuta, Sida rhombhofolia* and *Sida spinosa* are traditionally used as/in febrifuge, abortifacient, diuretic, dysentery, vomiting, gastric disorders, asthma, antipyretic, skin disease, diarrhea during pregnancy, rheumatism, neurological disorders, anti worm medication (6), anti-inflammatory, analgesic (7, 8), anti-malarial (9), anti-spasmodial (10), antibacterial (11), anti-diabetic (12), anti-hypertensive (13) and tonic properties (14).

The literature review revealed that extracts of roots and leafs of these species of *Sida* were known to have various pharmacological activities, while the fruits were screened only for its psychopharmacological activity which is extensively used in traditional systems of medicine for treatment of metabolic disorders. In light of above, in the present study, we aimed to explore the beneficial effects of *Sida tiagii* fruits as analgesics in different analgesic animal models.

MATERIALS AND METHODS

Drugs and chemicals

For phytochemical study, the required chemicals were procured from college chemical store supplied by Hi-media and Loba chemicals. The standard drugs, diclofenac sodium and pentazocine, were obtained as

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gift samples from Novartis and Ranbaxy, respectively. Xylene, n-hexane, ethyl acetic acid and ethanol were taken from SD Fine Chem. Ltd., Mumbai.

Plant materials and preparation of extracts

Sida tiagii was collected from the local field of Rajasthan (India) and identified by Dr. H.B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resource, (NISCAIR), India. The specimen was deposited to Herbarium, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar for future correspondence. The fruits were dried at 40 ± 1°C, grounded into a granulated powder and defatted with petroleum ether. The ethanolic extract was obtained by extracting 4 kg of defatted fruit powder with ethanol (95%) at 50°C for 72 h in Soxhlet apparatus followed by filtration and concentrated in rotaevaporator at 50 ± 5°C to its one third volume. The filtrate was partitioned with n-hexane (n-hexane extract, HS) and ethyl acetate (ethyl acetate extract; EAS) and the respective layers were separated out and dried on water bath at 30°C till dryness (HS – 32.23 g, EAS – 26.68 g). The residual ethanolic fraction (residual ethanolic extract, RES) was dried on water bath separately (104.10 g) and the extracts were stored at temperature below 10°C. The extracts were freshly prepared with 2% Tween 80 for preclinical pharmacological experiments (15).

Animals

Male Swiss mice (weighing 25–30 g, 3–4 month old) were obtained from Disease Free Small Animals House, Lala Lajpat Rai University of Veterinary Sciences, Hisar, India. The animals were maintained at controlled room temperature (27 ± 2°C) on a 12 h light/dark cycle with free access to food and water. All experiments were conducted between 9:00 a.m. and 17:00 p.m. The experimental protocol was approved by the Institutional Animal Ethical Committee, GJUS&T, and Hisar, India (Registration no. 0436).

Phytochemical analysis

Freshly prepared organic extracts were tested for the presence of alkaloids, steroids, triterpenoids, glycosides, tannins, flavonoids, carbohydrates and cardiac glycosides using standard procedures (3).

Pharmacological evaluation

Experimental design

Swiss mice divided in different groups were employed in the present study. Control group received distilled water; Standard group received pentazocine and test groups received HS, EAS and RES (300 and 500 mg/kg). Drugs were administered through *p.o.* route.

The acute toxicity and lethality

LD_{50} of various extracts of *Sida tiagii* in mice (n = 30) was estimated using the method described by Lorke (16). In stage one, animals received oral administration of 10, 100 and 1000 mg/kg (n = 5) of extracts (HS, EAS and RES) and observed for 24 h for number of deaths. Since no death occurred in any of the group in the first stage of the test, dose was increased (1600, 2900 and 5000 mg/kg) and administered to a fresh batch of animals (n = 5). No death was recorded within 24 h. The same test was repeated with different route of administration (*i.p.*) showing the same results. Thus, the oral and *i.p.* LD_{50} in mice was found to be greater than 500 mg/kg (29).

Acetic acid-induced writhing test

The analgesic effect was tested according to the method described by Shibata et al. (17). Abdomen writhing is a model of visceral pain and was produced by *i.p.* injection of 0.2 mL of 0.8% aqueous solution of acetic acid to each mouse 1 h after *p.o.* administration of test and standard compound. Immediately after the injection of acetic acid, each mouse was isolated in an individual observation box and the number of abdominal contortions per mouse was counted over a 30 min period (18, 19).

Tail immersion method

In this method, analgesia was assessed according to the method of Luiz et al. (20). Mice were held in position in a suitable restrainer with the tail extending out. Three to four cm area of the tail was marked and immersed in the water bath thermostatically maintained at 55°C. The withdrawal time of the tail from hot water (in seconds) was noted as the reaction time or tail flick latency. The maximum cut off time for immersion was 15 s to avoid the injury of the tissues of tail. The initial reading was taken immediately before administration of test and standard drugs and then 30, 60, 90, 120, 180 and 240 min after the administration. The criterion for analgesia was post-drug latency which was greater than two times the pre-drug average latency as reported by Janssen et al. (21). Tail flick latency difference or mean increase in latency after drug administration was used to indicate the analgesia produced by test and standard drugs (22, 23). Percentage of elonga-

tion was calculated using the following formula (24):

$$\text{Elongation (\%)} = \frac{\text{Latency (Test)} - \text{Latency (Control)}}{\text{Latency (Test)}} \times 100$$

Tail flick method

The tail flick latency was assessed by analgesiometer. The strength of the current passing through the naked nichrome wire was kept constant at 6 amperes. The distance between the heat source and tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut off reaction time was fixed at 10 s to avoid tissue damage (25, 26). Percentage of elongation was calculated using the following formula (24):

$$\text{Elongation (\%)} = \frac{\text{Latency (Test)} - \text{Latency (Control)}}{\text{Latency (Test)}} \times 100$$

Statistical analysis

All experimental data were expressed as the mean ± SEM. Statistical analyses were carried out by

Table 1. Preliminary phytochemical screening.

No.	Test	HS	EAS	RES
1	**Test for alkaloids**			
1.1	Mayer reagent	+	–	+
1.2	Dragendroff reagent	–	–	+
1.3	Wagner reagent	+	–	+
1.4	Hager reagent	+	–	+
2.	**Test for glycosides**			
2.1	Borntragers test	–	+	+
2.2	Keller-Killiani test	–	+	+
3	**Test for carbohydrates**			
3.1	Molisch test	–	+	+
3.2	Fehling test	–	+	+
3.2	Benedict test	–	+	+
4	**Test for sterols**			
4.1	Liebermann-Burchard test	–	+	+
4.2	Salkowski reaction	–	+	+
4.3	Hesse’s reaction	–	+	+
4.4	Herch-Sohn reaction	–	+	+
5	**Test for phenolic compounds and tannins**			
5.1	Ferric chloride test	–	+	+
5.2	Lead acetate test	–	+	+
6	**Test for proteins and amino acids**			
6.1	Millon test	–	+	+
6.2	Ninhydrin reagent	–	+	+
7	**Test for saponins**			
7.1	Foam test	–	–	–
7.2	Sodium bicarbonate test	–	–	–
8	**Test for flavanoids**			
8.1	Shinoda/Pew test	–	+	+
8.2	Ammonia test	–	+	+

(+) Present; (–) absent; (HS) n-Hexane extract of *Sida tiagii*; (EAS) Ethyl acetate extract of *Sida tiagii*; (RES) Residual ethanol extract of *Sida tiagii*

using one way ANOVA followed by Dunnett's *t*-test. The values of $p < 0.05$ were considered as significant.

RESULTS

Preliminary phytochemical screening of *Sida tiagii*

Preliminary phytochemical screening of *Sida tiagii* B. results in the presence of various compounds are shown in Table 1.

Acetic acid induced writhing:

The effect of EAS, HS and RES on writhing response in mice is shown in Table 2. EAS and RES (300 and 500 mg/kg) caused dose dependent inhibition of the writhing response induced by acetic acid. HS was found not significant as shown in Table 2.

Tail immersion test

Extracts obtained were subjected to evaluation for analgesic activity by tail immersion method using mice as animal model. Pentazocine (10 mg/kg) was taken as standard drug. EAS and RES extracts of seed of the *Sida tiagii* B. show more significant activity, while HS extract, did not show significant analgesic activity as compared to standard drug (Table 3).

Table 2. Effect of various extracts of *Sida tiagii* on acetic acid induced writhing in mice.

Time (min)	Control	HS (300 mg/kg)	HS (500 mg/kg)	EAS (300 mg/kg)	EAS (500 mg/kg)	RES (300 mg/kg)	RES (500 mg/kg)	Diclofenac sodium (15 mg/kg)
0–10	67 ± 1.8	66.6 ± 1.7	66.8 ±1.7	46.2 ±1.08*	35.0 ± 1.3*	52.0 ± 1.89*	38.0 ±1.2*	36.4 ±0.75*
10–20	42.4 ± 0.7	40.2 ± 0.86	39.8 ± 0.37	24.0 ± 1.5*	21.6 ± 0.5*	28.8 ± 0.8*	23.4 ± 1.4*	22.4 ± 0.60*
20–30	17.6 ± 0.75	17.0 ± 0.55	18.2 ± 0.58	11.8 ± 0.58*	12.8 ± 2.17*	13.2 ± 0.58*	13.2 ± 0.58*	10.8 ± 0.37*
Total	127 ± 3.25	123.6 ± 3.33	124.8 ± 2.65	82.0 ± 3.16	69.4 ± 2.17	94.0 ± 321	74.6 ± 3.18	69.6 ± 1.72

Values are the mean ± SEM from 6 animals in each group. One way ANOVA followed by Dunnett's t test. *$p < 0.05$ when compared to control group were considered as significant.

Table 3. Mean response of tail immersion method at various time interval.

Group	Dose	Mean basal time (s)	Mean response at various time intervals (min) (% of tail flick elongation)					
			30	60	90	120	180	240
Control (distilled water)	0.5 mL	6.33 ± 0.028	6.54 ± 0.05	6.58 ± 0.044	6.541 ± 0.034	6.45 ± 0.34	6.50 ± 0.104	6.42 ± 0.093
Standard	10 mg/kg	5.70 ± 0.087	8.801 ± 0.056* (25.69)	9.919 ± 0.034* (33.36)	10.14 ± 0.06* (35.55)	10.83 ± 0.078* (10.56)	10.88 ± 0.084* (40.25)	10.55 ± 0.10* (39.14)
HS	300 mg/kg	6.45 ± 0.045	6.55 ± 0.135 (0.15)	6.88 ± 0.046 (4.36)	6.85 ± 0.035 (4.54)	6.62 ± 0.056 (1.52)	6.58 ± 0.129 (1.22)	6.64 ± 0.02 (3.2)
HS	500 mg/kg	6.67 ± 0.066	6.59 ± 0.089 (0.75)	6.63 ± 0.076 (7.75)	6.70 ± 0.051 (2.38)	6.63 ± 0.048 (2.83)	6.59 ± 0.044 (1.09)	6.4 ± 0.074 (2.28)
EAS	300 mg/kg	6.36 ± 0.122	8.518 ± 0.046* (23.22)	9.938 ± 0.056* (33.78)	10.01 ± 0.046* (34.67)	10.56 ± 0.021* (38.92)	10.89 ± 0.05* (40.32)	10.54 ± 0.19* 38.12
EAS	500 mg/kg	6.53 ± 0.067	8.54 ± 0.087* (23.41)	9.68 ± 0.055* (32.02)	10.12 ± 0.087* (35.37)	10.42 ± 0.046* (38.09)	10.59 ± 0.09* (38.62)	10.40 ± 0.08* (38.26)
RES	300 mg/kg	6.84 ± 0.57	8.72 ± 0.064* (25.00)	9.77 ± 0.056* (32.65)	10.10 ± 0.031* (35.24)	10.66 ± 0.073* (39.49)	10.86 ± 0.008* (40.15)	10.39 ± 0.13* 38.16
RES	500 mg/kg	7.187 ± 0.076	8.87 ± 0.037* (26.27)	9.90± 0.028* (33.53)	10.05 ± 0.217* (34.92)	10.82 ± 0.076* (40.38)	10.95 ± 0.025* (40.63)	10.43 ± 0.17* (38.50)

Values are the mean ± SEM from 6 animals in each group. One way ANOVA followed by Dunnett's t test. *$p < 0.05$ when compared to control group were considered as significant.

Table 4. Mean response of tail flick method at various time intervals.

Group	Dose	Mean basal time (s)	Mean response at various time intervals (min) (% of tail flick elongation)					
			30	60	90	120	180	240
Control	0.5 mL	1.681 ± 0.099	1.788 ± 0.112	1.746 ± 0.053	1.855 ± 0.040	1.836 ± 0.028	1.82 ± 0.032	1.836 ± 0.079
Standard	10 mg/kg	2.415 ± 0.086*	5.77 ± 0.038* (69.01)	7.67 ± 0.082* (77.23)	8.306 ± 0.035* (77.67)	8.924 ± 0.151* (79.4)	8.58 ± 0.13* (78.76)	7.864 ± 0.168* (77.16)
HS	300 mg/kg	1.635 ± 0.098	1.898 ± 0.134 (5.79)	1.848 ± 0.093 (5.52)	1.951 ± 0.098 (4.87)	1.925 ± 0.073 (4.17)	1.858 ± 0.79 (1.63)	1.984 ± 0.069 (9.09)
HS	500 mg/kg	1.806 ± 0.038	1.93 ± 0.035 (7.78)	1.95 ± 0.072 (10.04)	2.018 ± 0.079 (8.07)	1.961 ± 0.065 (4.71)	1.856 ± 0.097 (3.72)	1.894 ± 0.097 (1.35)
EAS	300 mg/kg	2.113 ± 0.044*	4.535 ± 0.047* (60.52)	6.43 ± 0.028* (72.78)	7.372 ± 0.328* (74.89)	7.982 ± 0.043* (77.74)	8.297 ± 0.108* (77.93)	7.458 ± 0.39* (75.73)
EAS	500 mg/kg	2.07 ± 0.057*	4.55 ± 0.034* (60.87)	6.811 ± 0.049* (74.37)	7.210 ± 0.072* (74.27)	7.882 ± 0.045* (76.77)	8.326 ± 0.076* (78.12)	7.664 ± 0.069* (76.57)
RES	300 mg/kg	1.806 ± 0.105*	6.275 ± 0.027* (71.50)	7.78 ± 0.045* (77.56)	9.433 ± 0.49* (80.33)	9.73 ± 0.043* (81.13)	9.225 ± 0.894* (80.26)	8.718 ± 0.069* (79.33)
RES	500 mg/kg	2.16 ± 0.044*	6.68 ± 0.051* (73.23)	7.208 ± 0.411* (75.78)	9.66 ± 0.09* (80.79)	9.89 ± 432* (81.32)	9.158 ± 0.098* (80.11)	8.764 ± 0.069* (79.45)

Values are the mean ± SEM from 6 animals in each group. One way ANOVA followed by Dunnett's *t*- test. * p < 0 .05 when compared to control group were considered as significant.

Tail flick test

In tail flick test, both extract EAS and RES have significant analgesic activity and RES extract has more significant activity than diclofenac sodium but EAS has similar activity to diclofenac sodium. HS extract did not show significant result when compared to control group. (Table 4)

DISCUSSION

While the traditional use of herbal medicines is more or less obsolete today, it is still used for the remedy of diseases in a large number of patients throughout the world. Many plant metabolites are being successfully used in the treatment of variety of diseases. Even today, many people of the world population rely upon the plant resources for their medication (28). We investigated this time the fruits of *S. tiagii* for its analgesic activities. To investigate for analgesic activities of the seed extract of *S. tiagii* B. we used acetic acid-induced writhing tail immersion and tail flick methods. Intraperitoneal administration of acetic acid (0.8%) caused localized inflammation in mice due to the biogenesis of prostaglandins and leukotrienes. The biosynthetic prostaglandins, particularly prostacycline and prostaglandin E, have been reported to be responsible for the pain sensation due to intraperitoneal administration of acetic acid (29). Diclofenac sodium, like other non-steroidal anti-inflammatory drugs, inhibits the biogenesis of prostaglandins, thus inhibiting the writhing in experimental animals like mice. As the fruit extract of *S. tiagii* B. inhibits the writhing in mice, it is possible that the extract acts through the same mechanism of action as that of diclofenac sodium. Substance P is released in excessive quantities due to the stimulation of non myelinated C fibers of mouse's tail after the application of thermal heat. In case of acetic acid writhing test, prostacycline stimulates C fibers, whereas in thermal heat test C fibers are stimulated by thermal heat, serving as noxious stimuli. Narcotic analgesics like pentazocine are potential agonist of μ, κ and δ receptors. These receptors are specific for endogenous narcotics like endorphins, encephalin etc. After binding to these receptors, narcotic analgesics antagonize the action of substance P in the CNS by producing post-synaptic inhibitory action on interneuron, which processes the nociceptive information to be transmitted to the CNS. As our extract of *S. tiagii* B. showed significant analgesic activity in thermal heat method, it can be assumed that the extract could act by a central anti-nociceptive mode like that of pentazocine. In the present study, we investigated analgesic activity of two doses of various extract of *S tiagii* B. by acetic acid induced writhing method (22, 30, 31). The study indicated that at doses of 300 and 500 mg/kg body weight the

EAS and RES extracts caused significant inhibition of writhing. It was observed from the study that percent of inhibition of writhing can be increased by increasing the dose but all significant responses were not observed in HS extract. Results of two doses were also comparable with those of standard drug – diclofenac sodium. Similar analgesic activities were also observed in tail immersion and tail flick test. Doses of the seed extract significantly and dose dependently increased the elongation of tail flicking time in mice when compared to standard drug pentazocine. These observations are also consistent with those of Chevallier (31), when he observed significant analgesic activity in *S. tiagii* B. It is evident from the study that the fruit of *S. tiagii* B. extracted by ethyl acetate and ethanol exhibits significant analgesic effect in albino mice. We believe that further detailed advanced studies may be pursued in the future to explore the analgesic activities of the plant as well as its active constituents. The current study exhibited that oral administration of the various extracts of *Sida tiagii* B. produced dose dependent antinociceptive activity in two thermal models for nociception.

CONCLUSION

The data of the present study demonstrated that EAS and RES of *Sida tiagii* B. fruits produce a dose-dependent antinociceptive activity but not HS, as observed through different algesiometric tests. This antinociceptive effect may be partially related to the lipooxygenase and/or cyclooxygenase of the arachidonic acid cascade. Our results account for the analgesic effects of *Sida tiagii* B. and could be the reason for its wide use in folk medicine to treat different types of pain. Further study for the evaluation of main constituents responsible for this activity should be done by the researchers.

REFERENCES

1. Lingaraju G.M., Hoskeri H.J., Krishna V., Babu P.S.: Pharmacognosy Res. 3, 57 (2011).
2. Dawar R., Ali T., Qaiser M.: Willdenowia 25, 637 (1996).
3. Datusalia A.K., Kalra P., Narasimhan B., Sharma S., Goyal R.K.: J Health Sci. 54, 544 (2008).
4. Hassan G.A.U., Ahmad N.A.M., Alam M.T., Rizwani G.H.: Int. J. Pharmacognosy 37, 137 (1999).
5. Sahid T.M. Shahid R., Sheikhas A.S.: Pak. J. Pharm. Sci. 4, 145 (1991).
6. Parrotta J.A: Heatig plants of peninsular India. P. 483, CABI Publishing, Wallingford UK 1990.
7. Franzotti E.M., Santos C.V., Rodrigues H.M., Mourto R.H., Andrade M.R., Antoniolli A.R.: J. Ethnopharmacol. 72, 273 (2000).
8. Venkatesh S., Siva R.R.Y., Suresh B., Madhava R.B, Ramesh M.: J. Ethnopharmacol. 67, 229 (1999).
9. Karou D., Dicko M.H., Sanon S., Simpore J. Traore A.S.: J. Ethnopharmacol. 89, 291 (2003).
10. Banzouzi J.T., Prado R., Menan H., Valentin A., Roumentan C., Mallie M., Pelissier Y., Blache Y.: Phytomedicine 11, 338 (2004).
11. Oboh I.E., Akereler J.O., Obasuyi O.: J. Pharm. Res. 6, 809 (2007).
12. Raviknth V., Diwan P.V.: Phytother. Res. 13, 75-77 (1999).
13. Medeiros I.A., Santos M.R.V., Nascimento N.M.S. Duarte J.C.: Fitoterapia 77, 19 (2006).
14. Rastogi R.P. Malhotra B.N.: Compendium of Indian Medical Plants. Vol. 4, p. 674, Central Drug Research Institute, Lucknow 1985.
15. Farnsworth N.R.: J. Pharm. Sci. 55, 225 (1966).
16. Lorke D.: Arch. Toxicol. 53, 275 (1983).
17. Shibata S., Kamagai A., Harada M., Yano S., Saito H., Takahashi K.: U.S. Patent 43, 284 (1983).
18. Witkin L.B., Heubner F., Gardi F., Okeefe E., Sippitaletta S., Plummer A.J: J. Pharmacol. Exp. Ther. 133, 400 (1961).
19. Sastry A.V.S.: J. Chem. Pharm. Res., 3, 566 (2011).
20. Luiz D.D.S., Mirtes C, Sigrid L.J., Mizuekirizawa M., Cecilia G, Jrotin G.: J. Ethnopharmacol. 24, 205 (1988).
21. Janssen P.A.J., Niemegers C.J.E., Dony J.G.H.: Arzneimittelforschung 13, 502 (1963)
22. Ramabadran K.: J. Pharmacol. Methods 21, 21 (1989).
23. Vogel G.H.: Drug Discovery and Evaluation: Pharmacological Assays 2nd edn. Vol. 2, p, 697, Springer-Verlag, Berlin, Heidelberg, New York 2002.
24. Toma W., Graciosa J.S., Hiruma-Lima C.A, Andrade F.D.P.: J. Ethnopharmacol.. 85, 19 (2003).
25. Yoburn B.C., Morales R., Kelly D.D., Inturrisi C.E.: Life Sci. 34, 1755-1762(1984).
26. Dighe N.S.: Res. J. Pcog. Phychem. 1, 69 (2009).
27. Broneton J.: Pharmacognosy, Phytochemistry, Medicinal Plants, 2nd edn., pp. 330–387 Lavoisier, Adover 1995.

28. Berkenkopf J.W., Weichman B.M.: Prostaglandins 36, 693 (1988).
29. Chakraborty A., Devi R.K.B., Rita S., Sharatchandra K., Singh T.I.: Indian J. Pharmacol. 36, 148 (2004).
30. Hendershot L.C., Forsaith J.: J. Pharmacol. Exp. Ther. 125, 237 (1959).
31. Chevallier A.: The Encyclopaedia of Medicinal Plants. 1st edn., DK Publishing Inc., New York 1996.

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