Short Communication

Antitussive, expectorant activity of *Marsilea minuta* L., an Indian vegetable

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Abstract

Marsilea minuta L., an aquatic or sub-aquatic fern used as a vegetable, has wide applications in traditional/folk medicine in India and Bangladesh. In our study, we evaluated the antitussive, expectorant activity of *M. minuta* crude extracts. The antitussive activity of *M. minuta* methanol, ethyl acetate, and petroleum ether extracts was evaluated using ammonia and sulfur dioxide induced mice coughing. The expectorant activity was evaluated by the volume of phenol red in mice's tracheas. Extracts significantly increased mice's cough latent period and inhibited the frequency of cough induced by ammonia and sulfur dioxide, and improved tracheal phenol red output in expectorant evaluation. Methanol extract produced the highest activity in all tested models. Methanol extract at 500 mg/kg showed 59.5% and 55.8% inhibition in the number of coughing induced by ammonium liquor and SO_2, respectively, while it showed 89.3% increase in phenol red secretion at the same dose, which showed superior activity compared to other extracts. The present study provided evidence for *M. minuta* to be used as an antitussive and expectorant in Indian folk medicine.

Key words: Antitussive, expectorant, folk medicine, *Marsilea minuta*

INTRODUCTION

Marsilea minuta L., a common aquatic or sub-aquatic fern well-known as "susnisak" in Tripura, India, is a member of Marsileaceae family.[1,2] The leaves and shoots of the plant are commonly used as vegetable, and it finds extensive application in the treatment of cough and respiratory troubles. Juice of the fresh shoots and decoction of leaves are used to treat cough and other respiratory troubles by the people of India and Bangladesh.[2-4] The plant is widely used in different traditional and folk medicinal systems for its medicinal value, and recommended for the treatment of psychopathy, diarrhea, respiratory diseases, and skin diseases.[2,5,6] The young fronds of the plants are used to treat insomnia and mental problems, while regular eating of the plant is believed to exert favorable effects on hypertension, sleeping disorders, and headache. The plant is also recommended to treat spastic condition of leg and muscle, epilepsy, and migraine.[1,2,5-7] Leaves of *M. minuta* are prescribed by folk medical practitioners to treat diabetes and gastrointestinal disorders.[8,9] Investigation on its chemical constituents revealed that the plant contains marceline (an ester of 1-triacontanol and hexacosanoic acid), which is known to have sedative and anticonvulsant activity. In addition, different flavonoids and marsileagenin-A (a sapogenol) were isolated from the plant.[10,11] It has been reported that *M. minuta* possesses hypocholesterolemic,[12] anxiolytic,[13] antidepressant,[10] anti-aggressive,[14] antifertility,[15] antiamnestic, and antistress[16] activities.

The plant has been used as an alternative/complementary medicine for a thousand years.[17] Fruits and vegetables are a rich source of bioactive phytochemicals; therefore, regular and high intake of vegetables and fruits has been associated with a decrease in incidence of several chronic diseases like cancer, cardiovascular diseases, diabetes, etc.[18,19]

Coughing is one of the common symptoms related to several respiratory diseases such as asthma, bronchitis,

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pneumonia, etc., Though a number of synthetic drugs are available in the treatment of cough, the problem is that an effective therapy against coughing will inevitably bring side effects.[20] Northeastern India is blessed with a lot of floral diversity, abound with natural medicinal potentials. Owing to their medicinal and curing properties as well as the health-boosting constituents, vegetables with medicinal property are the choice of interest in both the traditional as well as modern versions of the health care system.

MATERIALS AND METHODS

Chemicals

Methanol, petroleum ether, ethyl acetate, ammonium hydroxide, sodium hydrogen sulfate, sulfuric acid, ammonium chloride, and sodium bicarbonate were obtained from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India) and SD Fine Ltd. (Mumbai, India). All other chemicals used in the study were obtained commercially and were of analytical grade.

Plant Materials

During the month of March/April 2011, the whole plant of *M. minuta* L. was collected from Tripura. The plant was identified and authenticated by Dr. B. K. Datta, Department of Botany, Tripura University, Tripura. A voucher specimen (TU/BOT/HEB/SS23072011b) was deposited at the herbarium of Plant Taxonomy and Biodiversity Laboratory, Tripura University.

Preparation of Extracts

Plants were collected and washed in running water to remove unwanted materials and dried under shade. The air-dried samples were powdered mechanically by using a grinder. Dried plant material was extracted using Soxhlet apparatus with methanol, ethyl acetate, and petroleum ether separately. The extracts were concentrated under reduced pressure. Dried methanol extract (MMM), ethyl acetate extract (EMM), and petroleum ether extract (PMM) were used to perform the study. The yield of MMM, EMM, and PMM was found to be 3.6, 3.9, and 3.2% w/w, respectively.

Experimental Animals

Healthy Albino mice (20-30 g) were used for the experiment. Animals were maintained under standard laboratory conditions at a temperature of 24 ± 1°C, with 12-h light–dark cycle. The animals had free access for water and food. Institutional Animal Ethical Committee (Reg. No. 1305/ac/09/CPCSEA) had given approval for the study.

Antitussive Activity

Ammonium liquor induced cough

Healthy mice were divided into eight groups: Control, MMM (250 and 500 mg/kg), EMM (250 and 500 mg/kg), PMM (250 and 500 mg/kg), and standard. Briefly, 1 h after oral administration of the test drug, each mouse was placed in a glass chamber and exposed to 0.3 ml 25% NH_4OH produced by a nebulizer for 45 s. Animal was monitored during ammonia exposure. The cough frequency and latent period of cough were recorded for 6 min.[21]

Sulfur dioxide (SO_2) induced cough

Healthy mice were divided into several groups and drug treatment was continued for 5 days. $NaHSO_3$ solution in water (500 mg/kg, 2.0 ml) was placed at the base of a specially deigned desiccator and 0.2 ml of sulfuric acid was added using a pipette which resulted in the formation of sulfur dioxide. After 15 s, each mouse was placed on the stage in the desiccator and exposed to SO_2 for 45 s. The mice were then removed and placed in a clear glass chamber for counting of bouts of cough for 5 min.[22]

Expectorant Activity

After 1 week of acclimatization, mice were divided into different groups as described earlier. Ammonium chloride (0.5%, 1.0 g/kg) was used as positive control. The treatment lasted for 5 days. After 30 min of last administration, the mice were injected with 5% phenolsulfonphthalein; 40 min later, animals were dissected to take out trachea and bronchial parts. Five percent $NaHCO_3$ was used to flush the inside of these parts three times, 0.5 ml for each time, which were collected and centrifuged to measure the absorbance of supernatant at the wavelength of 546 nm using UV–Vis spectrophotometer.[23]

Statistical Analysis

Experimental results were expressed as mean ± standard error of mean (SEM). P values < 0.05 were regarded as statistically significant. A one-way analysis of variance followed by Tukey's tests was used for the data analysis, using Statistical Package for Social Sciences (SPSS) version 10.0 software.

RESULTS

M. minuta demonstrated having antitussive effect in *in vivo* experiment by prolonging the latency and reducing cough [Table 1]. MMM and EMM at 500 mg/kg reduced the number of coughs induced by ammonium liquor by 59.5% and 54.8%, and significantly increased the latency period in a dose-dependent manner. Pretreatment with codeine, MMM, EMM, and PMM at a dose of 500 mg/kg for 5 days caused 69.1%, 55.8%, 56.8%, and 34.0% inhibition of SO_2-induced cough; lower dose of PMM failed to produce significant antitussive effect.

M. minuta extracts in multiple doses showed good dose-dependent expectorant activity [Table 2]. Methanol extract (500 mg/kg) exhibited high phlegm eliminating activity among the other tested extracts, but lower dose of PMM failed to produce significant activity. MMM (500 mg/kg) caused 89.3% increase in the

Table 1: Antitussive effect of *M. minuta* extract

Treatment	Dose (mg/kg)	Ammonium liquor induced cough			SO_2-induced cough	
		Latency period (s)	Number of cough	Percentage inhibition	Number of cough	Percentage inhibition
Control	10	10.44±2.33	49.83±5.32	-	34.33±3.32	-
MMM	250	18.02±3.03**	38.67±4.30*	22.4	22.00±2.85**	35.9
	500	26.33±3.39***	20.16±2.83***	59.5	15.16±2.44***	55.8
EMM	250	14.00±2.01*	40.00±4.77*	19.7	20.50±2.07***	40.3
	500	20.64±2.66***	22.50±2.90***	54.8	14.83±1.37***	56.8
PMM	250	9.89±1.99	45.83±4.83	8.0	28.16±2.55*	18.0
	500	15.12±2.44*	33.67±3.79*	32.4	22.67±2.21**	34.0
Codeine phosphate	30	33.22±3.83***	9.67±1.34***	80.6	10.16±1.44***	69.1

Values expressed as mean±SEM (n=6). *P<0.05, **P<0.01, and ***P<0.001 for comparison of treated groups with control

Table 2: Expectorant activity of *M. minuta* extracts

Treatment	Dose (mg/kg)	Absorbance	Concentration of phenol red (mg/ml)	Increase (%)
Control	-	0.061±0.01	0.28±0.19	-
MMM	250	0.082±0.01	0.46±0.22***	64.28
	500	0.090±0.02	0.53±0.20***	89.29
EMM	250	0.079±0.04	0.42±0.18***	50.00
	500	0.085±0.03	0.48±0.23***	71.43
PMM	250	0.062±0.01	0.31±0.11	10.71
	500	0.071±0.03	0.37±0.21*	32.14
Ammonium chloride	30	0.111±0.06	0.67±0.31***	139.28

Values expressed as mean±SEM (n=6). *P<0.05, **P<0.01, and ***P<0.001 for comparison of treated groups with control

concentration of phenol red, while EMM showed 71.43% increase at the same concentration, though the reference drug ammonium chloride produced far better activity than extracts, with 139.3% increase in the concentration of phenol red.

DISCUSSION

Antitussive animal models could be designed by mechanical stimulus, electrical stimulus, and chemical stimulus. In this experiment, chemicals like ammonium liquor and sulfur dioxide were used to induce cough. These models are widely used animal models for evaluating antitussive activity of a traditional candidate medicine.[21-23] The cough latency period, which is the time interval between exposure to ammonia hydroxide or SO_2 and appearance of cough, showed the potential of the MMM and EMM on delaying cough. The longer cough latency period showed potent effect of the extract on relieving cough and the less cough times exhibited its stronger antitussive effect.

Secretion of sputum is of benefit by dephlogistication of trachea and relieving cough. Expectorant drugs generally dilute or increase the sputamentum in the respiratory tract so that it could be expectorated easily with the ciliary movement. In the study, phenol red was discharged with sputum after a few minutes of its *i.p.* injection into mice because the agent was removed partly from trachea.[23] The extract showed significant expectorant activity.

Several researchers have reported the beneficial effect of saponin, flavonoid, and triterpenoid in the treatment of experimentally induced cough. In traditional medicinal system, a lot of plants have been used to treat cough. Systemic study of these plants showed the presence of saponin, alkaloid, terpenoids, and phenolic compounds.[24-26] In our previous studies, we have reported the presence of sapogenol, flavonoid, and triterpenoid in the plant.[10,11] In our previous study, we observed high content of phenolic and flavonoid compounds in the methanol extract.[27] It prompted us to lay a hypothesis that these constituents might have the antitussive and expectorant activities. However, due to the nature of multiple chemical constituents involved in the natural plants, as well as the multifactorial condition of respiratory diseases, it is very important to further separate chemical constituents from the methanol extract which are effective on the observed biological activities. Further studies are necessary to clarify the precise mechanism by which *M. mimuta* possesses the antitussive and expectorant activities.

CONCLUSION

To conclude, our study indicated that the methanol extract of *M. mimuta* demonstrated significant antitussive and expectorant effects. These effects are the important evidence for the traditional use of *M. mimuta* in the treatment of cough, respiratory disorders. The isolation of bioactive constituents and the mechanism of action explained for the observed activities have not been established, and thus further investigations need to be conducted.

REFERENCES

1. Sarker SK, Hossain EA. Pteridophytes of greater Mymensingh district of Bangladesh used as vegetables and medicines. Banglad J Plant Taxon 2009;16:47-56.

2. Sen S, Chakraborty R, De B, Devanna N. An ethnobotanical survey of medicinal plants used by ethnic people in West and South district of Tripura, India. J Forestry Res 2011;22:417-26.
3. Parihar P, Parihar L. Some pteridophytes of medicinal importance from Rajasthan. Nat Prod Rad. 2006;5:297-301.
4. Upreti K, Jalal JS, Tewari LM, Joshi GC, Pangtey YP, Tewari G. Ethnomedicinal uses of pteridophytes of Kumaun Himalaya, Uttarakhand, India. J Am Sci 2009;5:167-70.
5. Rahmatullah M, Kabir AA, Rahman MM, Hossan MS, Khatun Z, Khatun M. *et al*. Ethnomedicinal practices among a minority group of Christians residing in Mirzapur village of Dinajpur district, Bangladesh. Adv Nat Appl Sci 2010;4:45-51.
6. Rout SD, Panda T, Mishra N. Ethnomedicinal studies on some pteridophytes of Similipal Biosphere Reserve, Orissa, India. Int J Med Med Sci 2009;1:192-7.
7. Sharma NK. Ethnomedicinal studies on ferns and fern allies of Hadoti plateau, southeastern Rajasthan. Zoo's Print Journal 2002;17:732-43.
8. Anbarashan M, Parthasarthy N, Padmavathy A. Ethno-floristic survey in sacred groves, Pudukottai district. Tamil Nadu- India. J Med Plant Res 2011;5:439-43.
9. Thirumalai T, Kelumalai E, Senthilkumar B, David E. Ethnobotanical study of medicinal plants used by the local people in Vellore district, Tamilnadu, India. Ethnobotanical Leaflets. 2009;13:1302-131.
10. Bhattamisra SK, Khanna BK, Agrawal AK, Singh PN, Singh SK. Antidepressant activity of standardised extract of *Marsilea minuta* Linn. J Ethnopharmacol 2008;117:51-7.
11. Chakravarti D, Debnath NB, Mahato S, Chakravarti R. Structure of marsileagen: A new hexahydroxytriterpene from *Marsilea minuta* Linn. Tetrahedron 1975;31:1781-2.
12. Gupta RS, Kumar P, Sharma A, Bhardwaja TN, Dixit VP. Hypocholesterolemic activity of *Marsilea minuta* Linn. in gerbils. Fitoterapia. 2000;71:113-7.
13. Bhattamisra SK, Singh PN, Singh SK, Kumar V. Anxiolytic activity of *Marsilea minuta* Linn. J Herb Med Toxicol 2007;1:15-20.
14. Tiwari OP, Bhattamisra SK, Tripathi PK, Singh PN. Anti-aggressive activity of a standardized extract of *Marsilea minuta* Linn. in rodent models of aggression. Biosci Trends 2010;4:190-4.
15. Gupta M, Mazumder UK, Datta I, Battacharya S, Mukherjee S, Manikandan L. Studies of antifertility activity of *Marsilea minuta* Linn. Indian J Pharm Sci 2002;64:176-8.
16. Tiwari OP, Bhattamisra SK, Singh PN, Kumar V. Adaptogenic anti-stress activity of standardised extract of *Marselia minuta* L. Pharmacology online 2009;1:290-9.
17. Okoko T. *In vitro* antioxidant and free radical scavenging activities of Garcinia kola seeds. Food Chem Toxicol 2009;47:2620-3.
18. Hu FB. Plant-based foods and prevention of cardiovascular disease: An overview. Am J Clin Nutr 2003;78:544-51.
19. Kaisoon O, Siriamornpun S, Weerapreeyakul N, Meeso N. Phenolic compounds and antioxidant activities of edible flowers from Thailand. J Funct Foods 2011;3:88-99.
20. Wang D, Wang S, Chen X, Xu X, Zhu J, Nie L, *et al*. Antitussive, expectorant and anti-inflammatory activities of four alkaloids isolated from Bulbus of *Fritillaria wabuensis*. J Ethnopharmacol 2012;139:189-93.
21. Wang D, Zhu J, Wang S, Wang X, Ou Y, Wei D, *et al*. Antitussive, expectorant and anti-inflammatory alkaloids from Bulbus Fritillariae Cirrhosae. Fitoterapia 2011;82:1290-4.
22. Gupta YK, Katyal J, Kumar G, Mehla J, Katiyar CK, Sharma N, *et al*. Evaluation of antitussive activity of formulations with herbal extracts in sulphur dioxide (SO_2) induced cough model in mice. Indian J Physiol Pharmacol 2009;53:61-6.
23. Han N, Chang C, Wang Y, Huang T, Liu Z, Yin J. The *in vivo* expectorant and antitussive activity of extract and fractions from *Reineckia carnea*. J Ethnopharmacol 2010;131:220-3.
24. Guo C, Tang X, Dong X, Yang Y. Studies on the expectorant, antitussive and antiasthmatic properties of asterosaponin extracted from *Luidia quinaria.* Afr J biotechnol 2009;8:6694-6.
25. Parihar M, Chouhan A, Harsoliya MS, Pathan JK, Banerjee S, Khan N, *et al*. A review-cough and treatments. Int J Nat Prod Res 2011;1:8-18.
26. Wei Y, Xu F, Chen X, Chen X, Zhang G. Antitussive and expectorant effects of the flavonoid of *Cortex Mori*. J Shenyang Pharm Univ 2009;26:644-7.
27. Chakraborty R, De B, Devanna N, Sen S. Antitussive, expectorant activity of Marsilea minuta L., an Indian vegetable. J Adv Pharm Tech Res 2013;4:1-4.

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