

Anti-diarrheal Activity of Various Solvent Fractions of *Myristica fragrans* Seeds

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Abstract

The people of Bangladesh suffer in large numbers from diarrhea and dysentery (mostly caused by *Vibrio cholerae*, rotavirus, *Escherichia coli* and *Shigella*). The lack of proper hygienic living conditions, food and potable water system results in occurrence of diarrhea if not on an epidemic scale, then at least in individuals and families throughout the year. As the poor rural and urban slum people and even the affluent class in Bangladesh are used to alternative forms of treatment, it was of interest to evaluate various organic solvent extracts of *Myristica fragrans* seeds (origin of two spices, namely mace and nutmeg) for their anti-diarrheal activities. Diarrhea was induced in mice with castor oil. Of the four solvent extracts tested, namely methanol, petroleum ether, n-hexane, and chloroform, n-hexane fraction showed the highest activity at 400 mg per kg body weight with a reduction of diarrhea by 71.3%. The other fractions were also active at the two doses tested of 200 and 400 mg per kg body weight. Chloroform fraction demonstrated the lowest anti-diarrheal activity with 46.0% reduction of castor oil-induced diarrhea, when administered at 200 mg per kg body weight.

Keywords: Diarrhea; Dysentery; *Myristica fragrans*; Bangladesh

Introduction

Absence of proper disposal system of feces and other types of possible enteric pathogen-containing garbage along with lack of proper hygienic living conditions and unhygienic food and water is responsible for periodic large outbreaks of diarrhea and dysentery and occurrences of individual cases of diarrhea year round in Bangladesh. Disposal of feces in small water bodies like ponds and using the same pond water as a source of drinking water is a major cause for enteric diseases [1]. Among the various pathogens causing diarrhea and dysentery in Bangladesh are *Vibrio cholerae*, rotavirus, *Escherichia coli* and *Shigella* (including ciprofloxacin-resistant) [2-5]. On top of it, the general illiteracy and poverty can lead to lack of treatment resulting in especially high child mortality [6,7]. As a result, alternate modes of treatment are necessary, which uses more affordable and readily available forms of medicines. This is more so because modern doctors, clinics and hospitals are practically unavailable to the people of around 86,000 villages of the country.

Myristica fragrans Houtt. (Myristicaceae) is a tropical evergreen tree, whose seeds are well known and used in cuisine of a number of countries. The seeds are sources of two spices nutmeg and mace. Nutmeg is the seed kernel inside the fruit and mace is the covering or aril on the kernel. Aqueous extract of nutmeg reportedly caused inhibition of castor oil-induced diarrhea in mice and demonstrated anti-spasmodic effect on contractions induced by acetylcholine, nicotine and histamine in isolated guinea pig ileum [8]. Alcoholic extract of

nutmeg was found to be effective against rotavirus-induced calf diarrhea [9]. It was thus of interest to evaluate various solvent extracts of whole seeds of *M. fragrans* for their anti-diarrheal potential.

Methods

Plant material collection

Myristica fragrans seeds were collected from an herbal shop at Dhaka. The seeds were washed properly and then air dried for several days. The seeds were then grounded into a coarse powder using high capacity grinding machine.

Preparation of methanolic extract of grounded seeds

For preparation of methanol extract, 800g of the powder was extracted with 2.5 liters of methanol over 15 days with occasional stirring and shaking. The mixture was then filtered and methanol in the filtrate evaporated using a Rota evaporator. The final weight of the methanolic extract was 160g.

Solvent-solvent partitioning

10g of crude methanolic extract was dissolved in 10% aqueous methanol and sequentially extracted with petroleum ether, n-hexane and finally with chloroform [10].

Chemicals and drugs

Castor oil was purchased from Square Pharmaceuticals Ltd., Bangladesh. All other chemicals and reagents were of analytical grade.

Animals

Swiss albino mice were used in the present study, which weighed between 30 - 35g. The animals were obtained from International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). The animals were housed in the Animal House of Nutrition and Food Department of the University of Dhaka. The animals were acclimatized for three days prior to actual experiments. During this time, the animals were fed with mice chow (supplied by ICDDR,B) and water *ad libitum*. The study was conducted following approval by the Institutional Animal Ethical Committees of the University of Development Alternative and the University of Dhaka, Dhaka, Bangladesh [11].

Evaluation of anti-diarrheal activity

Fifty experimental mice were randomly selected, weighed and divided into ten groups consisting of five mice per group. Each group received a particular treatment. Prior to any treatment, individual mouse was weighed and doses of control and sample material(s) adjusted accordingly. Group 1 mice served as control and were administered vehicle only. Group 2 mice received a standard anti-diarrheal drug, loperamide at a dose of 10 mg per kg. Groups 3 and 4 received, respectively, methanol extract at doses of 200 and 400 mg per kg. Groups 5 and 6 received, respectively, petroleum ether extract at doses of 200 and 400 mg per kg. Groups 7 and 8 received, respectively, n-hexane extract at doses of 200 and 400 mg per kg. Groups 9 and 10 received, respectively, chloroform extract at doses of 200 and 400 mg per kg. All mice were fasted overnight. Vehicle, loperamide, and various extracts were administered to mice by gavaging 30 minutes prior to oral administration of 0.6 ml castor oil per mice. After 30 minutes following administration of castor oil, the number of diarrheal episodes in each mouse was monitored for four hours [12].

Statistical analysis

Experimental values are expressed as mean \pm SEM. The results were analyzed statistically by one way analysis of variance (ANOVA) followed by Dunnett's test using SPSS ver. 17. A value of $P < 0.05$ was considered to be statistically significant.

Results and Discussion

Four solvent extracts were tested for their anti-diarrheal activity against castor oil-induced diarrhea in mice. Of the four extracts tested, n-hexane fraction, at a dose of 400 mg per kg, gave the best result with a 71.3% inhibition of diarrheal episodes. The results are

shown in table 1. All extracts at all doses significantly inhibited diarrheal episodes, the percent of inhibition ranging from a low of 46% with chloroform fraction at 200 mg per kg to a high of 71.3% obtained with n-hexane. However, none of the extracts at the two doses tested were as efficient as the standard anti-diarrheal drug loperamide, which gave an inhibition of 83.9% in the number of diarrheal episodes at a dose of 10 mg per kg body weight. Nevertheless, the various extracts show promise and may with further studies, lead to isolation and identification of new drugs.

| Test samples | Number of diarrheal feces | % Reduction of diarrhea |
|---|---------------------------|-------------------------|
| | (Mean \pm SEM) | |
| Control (0.1 ml/ 10g) | 17.4 \pm 0.68 | 0 |
| Standard (Loperamide HCl) 10 mg/Kg | 2.8 \pm 0.37 | 83.91 |
| Crude methanol extract (Dose 200 mg/Kg) | 8.8 \pm 0.80 | 49.43 |
| Crude methanol extract (Dose 400 mg/Kg) | 7.2 \pm 0.73 | 58.62 |
| Petroleum ether fraction (Dose 200 mg/Kg) | 7.0 \pm 0.45 | 59.77 |
| Petroleum ether fraction (Dose 400 mg/Kg) | 6.6 \pm 0.93 | 62.07 |
| n-Hexane fraction (Dose 200 mg/Kg) | 7.4 \pm 0.24 | 57.47 |
| n-Hexane fraction (Dose 400 mg/Kg) | 5.0 \pm 0.32 | 71.26 |
| Chloroform fraction (Dose 200 mg/Kg) | 9.4 \pm 0.68 | 45.98 |
| Chloroform fraction (Dose 400 mg/Kg) | 8.2 \pm 0.58 | 52.87 |

Table 1: Effect of crude methanol extract and different fractions on castor oil-induced diarrhea in mice.

All administrations were made orally. Values represented as mean \pm SEM, (n = 5); *P < 0.05; significant compared to control animals.

The methanol extract of mace has been reported to have anti-bacterial activity against *Helicobacter pylori*; malabaricone C isolated from nutmeg also reportedly had anti-bacterial effects; it is also possible that various other components of the essential oil from the seeds may be responsible for the observed anti-diarrheal effects [reviewed in [13]].

Conclusion

The inner and outer parts of seeds of *M. fragrans* form two popular spices in Bangladesh, namely mace and nutmeg. As such, the seeds can form an important source for obtaining lead compounds or drugs effective against bacteria or virus-induced diarrhea and other enteric disorders. Although the active anti-diarrheal component(s) was not isolated and identified in the present study, further studies are ongoing to that effect.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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