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Determination of antiemetic, antimicrobial and anti-radical properties of two plants of Apiaceae family growing in Bangladesh

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ABSTRACT

Objectives: This study was conducted to investigate the antiemetic, antimicrobial and anti-radical properties of two plants of Apiaceae family named *Foeniculum vulgare* and *Centella asiatica*.

Materials and Methods: The antiemetic assay was carried out by using chick emetic model with minor modifications by calculating the mean decrease in the number of retching. The antimicrobial activity of the crude extract was performed by Disc Diffusion method. The anti-radical activity was determined by the 2, 2-diphenyl-2-picrylhydrazyl hydrate (DPPH) method.

Results: The anti-emetic activity of the selected two plants on young chicks revealed that these extracts have a less anti-emetic effect. The group of chicks treated with Chlorpromazine was found to have 38.4 retches as compared to the 60.4 retches of the control group, thus Chlorpromazine reduced the retches by 36.96%. The chickens treated with *Foeniculum vulgare and Centella asiatica* extracts inhibited the retches up to 7.01% and 15.67% accordingly. The minimum antimicrobial effect was found in these two crude extracts. Both of these extracts did not appear potent in terms of both zones of inhibition and spectrum of activity. In anti-radical activity test, the *Foeniculum vulgare and Centella asiatica* showed moderate free radical scavenging activity with IC_{50} value 240.39 µg/ml and 241.71% respectively while compared to that of the reference standard ascorbic acid.

Conclusion: The selected two plants of Apiaceae family possess less anti-emetic, minimum anti-microbial and moderate anti-radical activities.

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Keywords: *Foeniculum vulgare, Centella asiatica*, antiemetic, antimicrobial, anti-radical.

INTRODUCTION

A large amount of diverse bioactive compounds are produced from plant sources that provide not only basic nutrition but also health benefits. Various studies show that diet well provided with vegetables and fruits are advantageous for health.[1] Epidemiological evidence suggests that vitamins A, C, E, and phenolic compounds (flavonoids, tannins, and lignins) perform vital roles to delay aging, reduce inflammation, and prevent certain type of cancers.[2] The outstanding revolution of modern medicine actually comes from the natural sources and the medicinal plants play an important role from the beginning of this track. Estimation shows that about 80% of world's population use plants as medicine.[3] The medicinal plants are rich in secondary metabolites and essential oils of therapeutic importance. Only 150-200 plants are used in western medicine where about 10,000 to 15,000 plants are listed for medicinal significance worldwide.[4] Demand for the medicinal plant is increasing in both developing and developed countries due to growing recognition of natural products.[5,6]

Antiemetic agents work against emesis that is induced by side effects of various drugs usually general anesthetics, opioid analgesics, chemotherapy for cancer and motion sickness.[7] Synthetic antiemetic drugs also show side effects after long-term use. Thus natural products manufacturing has remarkably become a timely demand.[8] Antimicrobial susceptibility testing is applied for drug discovery and investigation of potential antimicrobial agents.[9] Microbial resistance is increasing day by day generating uncertainty about the future use of antimicrobial drugs. Plants extracts and phytochemicals with known antimicrobial properties can be significantly used for therapeutic treatments.[10,11] Oxygen-derived radicals or free radicals are generated constantly as part of normal aerobic life which is responsible for different diseases. Thus the study of free radicals has been

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considered a great deal of interest in recent years.[12] Free radicals are able to damage vital molecules like DNA, lipids, carbohydrates, proteins etc as they are highly reactive and unstable.[13] Antioxidants reduce the damaging ability of free radicals by donating an electron to a rampaging free radical and neutralizing it.[14] Phytochemicals that protect against free radical damage, accumulate in fruits and vegetables in high concentration. They act as natural antioxidants that limit and lessen the oxidative damage caused by reactive oxygen species (ROS).[14,15]

Apiaceae (Umbelliferae) family is one of the biggest plant families on the earth. This family comprises approximately 450 genera and 3700 species worldwide. Members of Apiaceae possess various compounds with many biological activities. Some of the main properties are ability to induce apoptosis, antibacterial, hepatoprotective, vaso-relaxant, cyclooxygenase inhibitory and antitumor activities.[16] In this study we have selected two different plants (*Foeniculum vulgare and Centella asiatica*) from Apiaceae family to evaluate their antiemetic, antimicrobial and anti-radical properties.

MATERIALS AND METHODS

Chemicals

All of the chemicals used in this study were of analytical grade.Copper sulfate was purchased from ScharlauChem-ie S.A. Barcelona, Spain. Metoclopramide hydrochloride was purchased from Square Pharmaceuticals Ltd. Dimethyl sulfoxide (DMSO), Polyoxy-ethylene sorbitan monooleate (Tween 80) and methanol were purchased from Merck, Darm-stadt, Germany.

Collection and proper identification of plants sample

For this present investigation *Foeniculum vulgare (F. vulgare) and Centella asiatica (C. asiatica*) were collected from Dhaka District, Bangladesh and was identified by Bangladesh National Herbarium, Mirpur (Accession no: 37667 and 38976 respectively)

Drying and grinding of plant materials

The collected plant parts (seeds) were sun-dried for one week. The plant parts were ground into a coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis commenced.

Extraction of Plant materials

About 400 gm of powered material (each plant individually) was taken in a clean, flat-bottomed glass container and submerged in 2100 ml of 100% 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 clean, white cotton material. Then it was filtered through cotton plug. The filtrate (methanol extract) obtained was evaporated under ceiling fan and in a water- bath until dried. It rendered a gummy concentrate of reddish black color. The gummy concentrate was nominated as crude extract of methanol.

In Vivo Antiemetic Activity

Chick emetic model[17] was used to assay the antiemetic potency of the plants with minor modifications by calculating the mean decrease in the number of retching. 2-4 days young male chicks weighing from 30-32 gm were collected from a local poultry store. After 24 hrs fasting, the antiemetic activity was evaluated. All chicks were kept under laboratory conditions at room temperature with 12 hour light and dark cycles. All animal experiments were carried out in accordance with the acts of the Ethical Committee of Chittagong University. After 24 hours fasting, the antiemetic activity was evaluated. The chicks were divided into three groups of five chicks each and each chick was kept in a large beaker at 25°C for 10 minutes. The extracts of each plant dissolved in 0.9% saline containing 5% DMSO and 1% Tween 80 and administered at a dose of 150 mg/kg orally and a volume of 10 ml/kg to the test animal on the basis of their body weights. Control group received only saline (0.9% NaCl). After 10 minutes copper sulphate was administered orally at 50 mg / kg body weight, then the number of retching was observed during next ten minutes. Chlorpromazine was used as a standard drug (150 mg/kg .b.w).

The antiemetic effect was assessed as the decrease in thenumber of retches in the treated group in contrast to the control. The inhibition (%) was calculated as follows:

$$\text{Inhibition (\%)} = [(A-B)/A] \times 100$$

Where A is the frequency of retching of control group and B is the frequency of retching of the treated group.

Antimicrobial Screening

The crude extracts of the plants were tested for antimicrobial activity by disc diffusion method.[11] The bacterial and fungal strains used for the experiment were collected as pure cultures from the Microbiology research laboratory, Chittagong University, Bangladesh. Both gram positive and gram-negative organisms were taken for the test.

Staphylococcus aureus was taken as a gram-positive organism and *Escherichia coli, Pseudomonas aeruginosa, Enterobacter and Vibrio cholera* were taken as gram-negative organisms. Nutrient agar medium (DIFCO) was used in the present study for testing the sensitivity of the organisms to the test materials and to prepare fresh cultures.

The antimicrobial potency of the test agents was determined by their activity to prevent the growth of the microorganisms surrounding the discs by measuring the diameter of the zones of inhibition in millimeter with a transparent scale. In order to avoid any type of cross-contamination by the test organisms, the antimicrobial screening was done in Laminar Hood and all types of precautions were highly maintained. Petridishes and other glassware, Micropipette tips, cotton, forceps, blank discs etc. were also sterilized.

Anti-radical activity

The free radical scavenging activities (antioxidant aptitude) of the plant extracts on the stable radical 1, 1-diphenyl-2-picrylhydrazyl (DPPH) were estimated by the method of Brand- Williams.[18] Being a stable free radical, DPPH has delocalized spare electron over the molecule offering a deep violet color. If a substance with the potential to donate a proton is mixed with DPPH solution, the violet color vanishes from sight representing a reduced form of DPPH.[19]

Table 1 **% of inhibition of Retches for *Foeniculum vulgar* and *Centella asiatica* Extract**

Drug/dose	No. of retches	% inhibition
Control (10ml/kg)	60.04 ± 13.67	00
Chlorpromazine (150 mg/kg)	38.4±10.19	36.96%
Foeniculum vulgare Seeds (150 mg/kg)	54.8±7.18	7.01%
Centella asiatica leaves (150 mg/kg)	49.2±4.22	15.67%

Table 2 **Antimicrobial activity of test samples of *F. vulgare* and *C. asiatica***

Test microorganisms	Diameter of zone of inhibition (mm)± SEM		
	MCE (*F. vulgare*)	MCE (*C. asiatica*)	Ciprofloxacin
Gram positive Bacteria			
Staphylococcus aureus	3.83±0.37	4±0.33	16.00±0.57
Gram negative Bacteria			
Escherichia coli	2.58±0.61	1±0.02	30.66±0.33
Pseudomonas aeruginosa	1.63±0.12	2±0.57	35.66±0.88
Vibrio Cholera	4.11±0.21	5±0.88	11.00±0.57
Enterobacter	4.89±0.64	3±0.34	30.66±0.33

MCE: Methanolic crude extract of the plants (400 µg/disc)

At first 0.3 mg DPPH (0.004%w/v) is weighted accurately and dissolved in 15 ml methanol to make the concentration 20 µg/ml. 100 mg of dried sample extract (each plant individually) was dissolved in 10 ml of methanol for the concentration of sample solution 10 µg/ml. 21 test tubes were taken and each of this labeled for 5ml, in which 10 test tubes for different conc. of sample solution, 10 test tubes for different conc. of standard solution and 1 test tubes for blank which was filled with 3 ml DPPH solution and 2 ml of methanol. In each test tube (Except blank test tube) 3ml of DPPH solution (20 µg/ml) was taken and then mixed at different concentration (500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 16.625 µg/ml, 7.813 µg/ml, 3.906 µg/ml, 1.953 µg/ml and 0.997 µg/ml) of 2 ml sample solution (10 µg/ml). After 30 min reaction period at room temperature in dark place the absorbance was taken at 517 nm against methanol as blank by UV spetrophotometer. Control sample was prepared by using same conc. of ascorbic acid instead of sample solution (plant extract). The absorbance was recorded and percentage scanvenging (IC_{50}%) was determined using the following equation and was compared with ascorbic acid which was used as a standard.

(IC_{50}%) = (1 – Absorbance of test sample / Absorbance of control) X 100

Where Absorbance of the control means blank absorbance (containing all reagents except the test material).

Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

Statistical Analysis

All numerical data are expressed as the mean ± SEM (standard error of the mean) and Statistical analysis was carried out usingt-test and differences between means were considered to be significant when $p < 0.05$.

RESULT

In Vivo Antiemetic Activity

Number of retches recorded for crude methanolic extracts of *Foeniculum vulgare, Centella asiatica* and standard drug are given in table below –

Antimicrobial Screening

The minimum antimicrobial effect was found in these two crude extracts. Both of these extracts did not appear potent in terms of both zones of inhibition and spectrum of activity (Table 2).

Antiradical screening

The methanol extract of plant of *F. vulgare* and *C. asiatica* were tested for Free radical scavenging

Table 3 IC_{50} value of methanol soluble fraction of *F. vulgare*, *C. asiatica* and ascorbic acid

Serial	Sample	IC_{50} (µg/ml)
1.	Methanol soluble fraction (*F. vulgare)*	240.39
3	Methanol soluble fraction (*C. asiatica)*	241.71
2.	Ascorbic acid	27.23

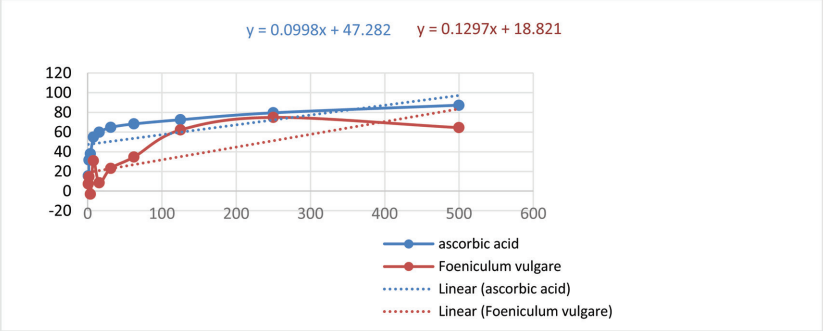

Figure 1 IC_{50} value for extract of *F. vulgare* and ascorbic acid standard using DPPH

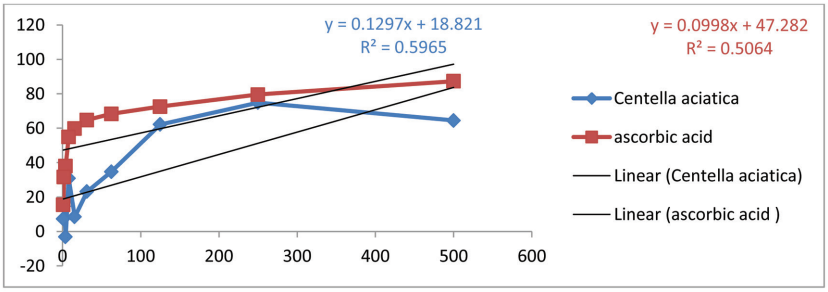

Figure 2 IC_{50} value for extract of *C. asiatica* and ascorbic acid standard using DPPH

activity. Absorbance of different concentration of methanol extract of mentioned plants are given below.

DISCUSSION

The anti-emetic activity of *C. asiatica* leaves and *F. vulgare* seeds on young chicks revealed that these extracts have a less anti-emetic effect. After administration of a dose of 150 mg/kg Chlorpromazine and the extracts of the plants, the numbers of retches were reduced. The group of chicks treated with Chlorpromazine was found to have 38.4 retches as compared to the 60.4 retches of the control group, thus Chlorpromazine reduced the retches by 36.96%. The chickens treated with the extracts of *C. asiatica* and *F. vulgare* (individually) inhibited the retches up to 15.67% and 7.01% respectively. Therefore, both of the extracts inhibited emesis to an extent not greater than Chlorpromazine at 150 mg/kg. On the basis of these results, it may be concluded that extracts of the plants have less anti-emetic potential and are comparable with that of Chlorpromazine (the reference drug).

The present study revealed minimum antimicrobial activity of the two plant extracts against *staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa*, *Vibriocholera*, *Enterobacter.* The methanolic crude extracts of these plants didn't significantly inhibit the growth of above mentioned microorganism. The plants (*Foeniculum vulgare and Centella asiatica*) from Apiaceae family exert such moderate antimicrobial properties due to their phytochemicals content. Different types of phytochemicals are responsible for several pharmacological activities like antimicrobial, antiemetic and antiemetic activities.[20]

Natural antioxidants are generally used against oxidative stress damage in a view to protect human being. The IC_{50} value is used as an indicator to measure the toxicity exhibited by the plant crude extracts. It is the concentration of drug required to restrain the growth and proliferation of the cells by 50%. [21] In the present investigation the methanol extract of plant of *C. asiatica* leaves and *F. vulgare* were tested for Free radical scavenging activity to determine the potential of anti-radical property. The IC_{50} value of the extract of *C. asiatica* and *F vulgare* were observed 241.71 µg/ml and 240.39 µg/ml which was not appeared as potent antioxidant to inhibit cell proliferation.

CONCLUSION

In the context of the above discussion, it can be revealed that the crude extract of *C. asiatica* and *F. vulgare* possess minimum anti-emetic and anti-microbial activities and moderate anti-radical properties. However, further studies on this plant extract require to find out the bioactive compounds which are mainly responsible for these pharmacological activities.

CONFLICT OF INTEREST

Authors has no conflict of interest.

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