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Antioxidant, antimicrobial, cytotoxic and analgesic activities of ethanolic extract of *Mentha arvensis* L.

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PEER REVIEW

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Comments

This is a valuable research work in which authors have made the study interesting by evaluating the antioxidative, analgesic, cytotoxic and antibacterial effects of *M. arvensis* L. extract in association with phytochemical screening. Materials and methods are well planned. Findings are attention-grabbing and the discussion section contains scientifically interpretation.
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ABSTRACT

Objective: To investigate potential antioxidant, antimicrobial, cytotoxic and analgesic activities of ethanolic extract of *Mentha arvensis* L. in different *in vivo* and *in vitro* experimental models.
Methods: *In vitro* DPPH radical scavenging assay was used to evaluate the antioxidant activity of the plant extract. *In vivo* analgesic activity was carried out by acetic acid-induced writhing test in Swiss albino mice. All studies in mice were undertaken at the doses of 250 and 500 mg/kg body weight. Antibacterial activity was studied by disk diffusion assay against some Gram-positive and Gram-negative bacterial strains. Brine shrimp lethality assay was used to investigate cytotoxicity effects of the plant extract.
Results: The extract showed free radical scavenging activity in the DPPH assay (IC_{50}~41 μg/mL) compared to the standard antioxidant ascorbic acid (IC_{50}~19 μg/mL). The extract also produced prominent antimicrobial activity against *Salmonella typhi*, *Salmonella paratyphi*, *Shigella boydii*, *Streptococcus pyogenes* and *Streptococcus aureus* compared to standard drug kanamycin at the dose of 30 μg/disc. The extract exhibited lethality against the brine shrimp nauplii with the LC_{50} values of 40 μg/mL, and also 90% mortality (LC_{90}) value was found to be 160 μg/mL. In analgesic test, the extract demonstrated statistically significant (P<0.01) analgesic effect in acetic acid induced writhing in white albino mice at both dose levels.
Conclusions: These results suggest that the ethanolic extract of *Mentha arvensis* L. has potential antioxidant, antibacterial, cytotoxic and analgesic activities that support the ethnopharmacological uses of this plant.

KEYWORDS
Antioxidant, Antibacterial, Cytotoxic, Analgesic, *Mentha arvensis* L.

1. Introduction

Mentha arvensis (*M. arvensis*) belonging to the family of Lamiaceae, is a small to moderate sized perennial herb, commonly known as pudina, corn mint or wild mint in Bangladesh and India. It is widely cultivated in Bangladesh, Nepal, India, Srilanka, Thailand, and Japan for its use as a food seasoner, household remedy, and industrial purposes. The plant has been reported to possess a large number of different chemicals like α-menthol, neomenthol, menthofuran, d-menthone, isomenthol, isomnethone, menthylacetate, cineol, phellandrene, p-cymene, aromadendrene, limonine, piperitone, carvomenthone, pinene, carvacrol, α-pinene, α-phellandrene, dipentene, cadinene, thujone, menthofuran, carvone, linalool, linalyl acetate and piperitenone oxide which are used

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in pharmaceutical, food, flavour, cosmetics, beverages and allied industries[1-4]. The plant leaf and oil contain acetaldehyde, amyl alcohol, methyl esters, limonene, β-pinene, β-phellandrene, cadinene, dimethyl sulphide, and traces of α-pinene, sabinene, terpinolene, *trans*-ocimene, g-terpinene, fenchene, α-thujone, β-thujone, citronellol and luteolin-7-O-rutinoside[5]. It also possesses the flavonoids like quercetin, menthoside, and isorhoifolin, vitamin K, eugenol and thymol[2]. According to several reports the plant contains 90% mint oil. It contains monoterpenes (menthone, menthofuran, methyl acetate cineole and limonene), sesquiterpenes (viridiflorol), flavonoids (luteolin, menthoside, isorhoifolin, rutin hesperidin), phenolic acids (caffeic acid, chlorogenic and rosmarinic), triterpenes (squalene, a-amyrin, urosolic acid and sitosterol), phytol, tocopherols, carotenoids, choline, betaine, cyclenes, rosmarinic acid, tannin and minerals[6-8]. More recently, linarin (acacetin-7-O-β-D-rutinoside) was extracted from the flower of the plant[6].

M. arvensis L. is used as a carminative, anti-spasmodic, anti-peptic ulcer agent, and has been given to treat indigestion, skin diseases, coughs and colds in folk medicine. Different parts of the plant have been reported to possess diverse medicinal properties. The leaves are stimulant, acrid, thermogenic, antispasmodic, antihelmenthic, anodyne, vulnerary, deodorant, sudorific, dentrific, febrifuge, contraceptive, carminative, digestive, expectorant, cardiotonic, diuretic and hepatalgia[7]. It is also used for the treatment of liver and spleen disease, jaundice and asthma. The infusion of leaves is used to treat indigestion, rheumatism, infantile troubles, vomiting in pregnancy, hysteria and as remedy for inflamed joints[9]. The plant is used in small amount in the mixtures of lotions, ointments and creams to treat skin disorders. It also acts as an antipruritic, a counterirritant, an antiseptic, a stimulant and an anaesthetic in treating dermatological cases. The entire plant, apart from the root, is used to treat coryza, fever, headache, rhinitis, cough, pharyngitis, arthralgia, neuralgia, abdominal colic, nausea, vomiting, dyspepsia, diarrhea and prurigo. It is also claimed to be an emmenagogue[7]. The dried plant is used as an antiseptic, carminative, stomachic, refringent, stimulant, emmenagogue and diuretic[7]. The aerial part is used in Chinese medicine as a cooling remedy for colds, influenza, headache, sore throat and eye inflammation. It is also used as a liver stimulant[7].

Recent investigations have confirmed that the plant extract possesses hepatoprotective, anti-oxidant, anti-allergic, anti-inflammatory, sedative-hypotonic and antimicrobial effect[7,8-10]. The traditional uses claim that *M. arvensis* L. is a potential folk medicine but very few phytochemical and biological works have been conducted on this plant. This issue is particularly crucial for medicinal interest and, to the authors' knowledge, has not been resolved thus far. The present experimental study was carried out to evaluate the pharmacological basis for the use of the plant in folk medicine by using established scientific method.

2. Materials and methods

2.1. Plant material

The plant *M. arvensis* L. was collected from Badargonj Upazilla of Rangpur district, Bangladesh during the month of January 2011 at day time. The plants were mounted on paper and the sample was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (DACB Accession Number-3678).

2.2. Extraction preparation

The collected plants were washed by fresh water, cut into small pieces, and shed dried for two weeks. The dried plant material was grounded into fine powdered form. The plant extract was collected by cold extraction method by taking 200 mg powders in 700 mL ethanol in a glass container for 14 d. The extract was separated from the plant debris by filtration using Whatman filter paper. The extract was concentrated in evaporation (initially by open air and finally by water bath) process. The amount of yield in the extract was 8.77%.

2.3. Animals

Swiss albino mice of either sex (20-29 g body weight) were collected from animal resources. Mice of random sex (Swiss-webstar strain, 19-40 g body weight) were collected from animal resources branch of the International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) and were used for the experiments. The animals were kept at animal house (Pharmacy Discipline, Khulna University, Khulna) for adaptation after collection under standard laboratory conditions (relative humidity 55%-65%, room temperature (25±2) °C and 12 h light: dark cycle) and fed with standard diets (ICDDR, B formulated) for period of 14 d prior to performing the experiments.

2.4. Chemicals

Diclofenac sodium was collected from Square Pharmaceuticals Ltd., Bangladesh. Glacial acetic acid and ascorbic acid were purchased from Sigma Chemicals, USA. All other chemicals were of analytical grade.

2.5. Phytochemical group test

The preliminary phytochemical group test was carried out by following standard procedure[1]. The extract was screened for the presence of alkaloids, reducing sugar, flavonoids,

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