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Chemical composition, total phenolic and flavonoid contents, and *in vitro* antimicrobial and antioxidant activities of crude extracts from red chilli seeds (*Capsicum frutescens* L.)

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Abstract

The objectives of present study were to assess the antimicrobial and antioxidant potential of *Capsicum frutescens* L. seeds and to characterize the chemical constituents of the crude extracts. The *n*-hexane and chloroform extracts were analyzed using gas chromatography–mass spectroscopy (GC–MS), which showed the presence of many biologically important volatile constituents, including heterocyclic compounds, β -diketones, hydrocarbons, long chain aliphatic carboxylic acids, and their derivatives, such as esters, hydroxy ester, and aromatic compounds. The amounts of the total phenolic content and the total flavonoid content in same the extracts were in the ranges of 7.95–26.15 gallic acid equivalents (GAE mg/g) and 4.64–12.84 rutin equivalents (RU mg/g) of dry weight of extract, respectively. In the determination of the *in vitro* antimicrobial activity, seed extracts prevented the growth of most of the tested pathogens by forming significant inhibition zones. The inhibitory activity was especially remarkable (inhibition zone ≥ 13 mm) against *Pesudomaonas aeruginosa*, *Klebsilla pneumonae*, *Staphylococcus aureus* and *Candida albicans*. During the evaluation of the *in vitro* antioxidant activity *via* DPPH assay, *n*-hexane and chloroform extracts showed 26.9% and 30.9% free radical scavenging abilities, respectively, at the concentration of 1 mg/mL. Considering these results, *C. frutescens* seeds can be used as a source of novel antimicrobial and antioxidant compounds.

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Keywords: Capsicum frutescens L.; GC-MS; Total phenolic content; Total flavonoid content; Antimicrobial activity; Antioxidant activity

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1. Introduction

Capsicum peppers are among the oldest cultivated plants in the world [1]. This genus is indigenous to Central and South America from pre-Colombian times and is in the nightshade family Solanaceae. Presently, this genus is believed to consist of 27 species, five of which are domesticated and used as fresh vegetables and spices, along with approximately 3000 varieties [2]. Widespread

geographic distribution of *Capsicum annuum* and *Capsicum frutescens* from the New World to other continents occurred in the sixteenth century *via* Spanish and Portuguese traders; soon afterwards, they became an integral part of food habits of several countries, including India.

The dried ripened red pod of *C. frutescens* is known to offer the pepper, which is used as a spice to flavour dishes worldwide. In addition to acting as a flavouring and colouring agent, this fruit also has ethnomedicinal prestige and is used to treat a variety of human ailments. Red chilli has been used as an alternative medicine for the treatment of inflammation, diabetes, low back pain and acute tonsillitis [3–5]. Moreover, capsicum plaster containing powdered capsicum and capsicum tincture has been used in Korean hand acupuncture to reduce postoperative nausea, vomiting and sore throat [6,7]. Chilli was an important plant in traditional Mayan medicine to treat various ailments, such as sore throat, earache and skin care [8].

The substances responsible for the pungency of *C. frutescens* pods are the capsaicinoids alkaloids, which are known for their pharmacological, neurological and dietetic effectiveness. These substances have significant antibiotic activity and the ability to reduce the cholesterol level in blood when used at low levels in the regular diet [9].

Although numerous studies have been reported in the literature to justify the medicinal importance of chilli peppers in food, it is notable that the chemical composition and extent of different bioactivities vary considerably with the species and cultivar investigated, along with the extraction conditions used in the experiments. Central India, especially the Malwa region of Madhya Pradesh, is well known for its hot and spicy cuisine; however, to the best of our knowledge, no study has been performed on the locally grown cultivar of C. frutescence to understand the role of red chillies in maintaining public health. Thus, in continuation of our work on the identification of pharmacologically active constituents from locally grown plants [10–12], the present study was planned. The goals of the present study on the seeds of dried pods of C. frutescens were the following: (i) to investigate the chemical composition of comparatively less explored low polar n-hexane and chloroform extracts, (ii) to evaluate its in vitro antimicrobial activity against common food borne pathogens, (iii) to determine the total phenolic and flavonoid content, and (iv) to estimate the antioxidant activity by DPPH radical scavenging assay.

2. Materials and methods

2.1. Chemicals

The organic solvents used in the experiments were of analytical grade and purchased from Qualigen Chemicals, India. The Muller Hinton agar culture medium, nutrient agar, Sabouraud dextrose agar medium and antibiotics discs used in study were procured from Himedia Laboratories Pvt. Ltd., Mumbai, India. The other chemicals used were of analytical grade and obtained from Merck, India.

2.2. Plant sample

Air-dried pods of *C. frutescens* were collected from the local spice market of District Ratlam, India. Plant samples were duly authenticated by Dr. V. Gupta, Taxonomist, Department of Botany, Govt. Arts & Science College, Vikram University, Ratlam, India, where a voucher specimen was also deposited for future reference.

2.3. Extraction procedure

The plant samples were washed several times with tap water and finally with distilled water to remove dust. The samples were dried under shade at room temperature. The seeds were separated from dried pods by crumbling and then screening. The shade dried seeds were further ground by means of a mechanical blender (Bajaj GX10, India) to fine powder. One hundred grams of the seed powder was sequentially extracted for 3 days with each solvent n-hexane (500 mL \times 3) and chloroform (500 mL \times 3) using a Soxhlet apparatus over a water bath. The extracts obtained were filtered through Whatman No. 1 filter paper and then evaporated to dryness by using a rotary evaporator (Buchi, Switzerland). The final crude extracts were collected in an airtight container and then refrigerated at 4 ± 2 °C until further use.

2.4. Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analysis of extracts was performed using a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett-Packard 5970A Mass spectrometer detector (MSD). Compounds were separated on a fused silica capillary column with a column length of 25.0 m, an internal diameter of 0.32 mm and a film thickness of 0.25 μ m. The temperature of the injector was 250 °C, and 2 μ L of

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