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Original article

Isolation and characterization of bioactive components from *Mirabilis jalapa* L. radix

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ABSTRACT

The present investigation was carried out to isolate and characterize bioactive components from *Mirabilis jalapa* L. radix (紫茉莉根 zǐ mò lì gēn). Thin-layer chromatography was used for the separation of spots from fractions of the crude extract. Separated spots were collected for identification of their activities. Free-radical scavenging activity was evaluated by spraying thin-layer chromatography plates (spotted with fractions) with 0.2% of 2,2-diphenyl-1-picrylhydrazyl solution. Activity against human pathogens such as *Staphylococcus aureus* and *Candida albicans* were determined using the agar diffusion method. Potential spots were subjected to infrared (IR) analysis and gas chromatography for characterization. Two spots (5F1 and 1F3) showed free-radical scavenging activity. The 1F3 spot was active against both *S. aureus* and *C. albicans,* whereas the 5F1 spot was active against *S. aureus* only. IR spectral analysis indicated that 5F1 spot to be a triterpenoid. Using IR spectral analysis and an IR library search, the 1F3 spot was identified to be a flavone, which may have a hydroxyl group in ring "A" of the flavone nucleus. Our results indicated that the 1F3 and 5F1 spots are potential free-radical scavengers. Both 1F3 and 5F1 exhibited antimicrobial activity. IR spectral analysis coupled with an IR library search indicated 1F3 and 5F1 to be a flavone and a triterpenoid, respectively.

1. Introduction

Mirabilis jalapa L. (Nyctaginaceae; 紫茉莉根 zǐ mò lì) is a traditional medicine widely used in many parts of the world for the treatment of various diseases. It is a perennial herb, which reaches a height of 50–100 cm from the tuberous root. It produces beautiful flowers that usually open at around 4 o'clock in the afternoon, and hence, its common name is *four o'clock* plant. It is a popular ornamental plant grown worldwide for the beauty of its flowers (which can be white, pink, yellow, or multicolored), which have a sweet fragrance.[1] Traditionally, the tuberous root of *M. jalapa* has been used for treatment of carbuncles (a skin infection caused by *Staphylococcus aureus*).[2]

Phytochemical investigation of the extracts from this plant showed that it is rich in many active compounds including triterpenes, proteins, flavonoids, alkaloids, and steroids. Alanine, alpha-amyrins, arabinose, beta-amyrins, campesterol, daucosterol, and dopamine were the other compounds reported from extracts of this plant.[1] Gas chromatography (GC)/mass spectral analysis of dichloromethane and methanol extracts of *M. jalapa* tubers indicated that oleic acid and beta-sitosterol, respectively, were the major compounds present. Liquid chromatography/mass spectroscopy analysis of the aqueous extract of the tuberous root of the plant showed a high content of flavonols. Phenolic acids such as ferulic acid and caffeic acid were also detected in the plant extracts. It has been also reported that the water extract of *M. jalapa* tubers, containing higher amounts of flavonoids, exhibits antimicrobial and antioxidant activities.[3] In comparison with terpenoid and flavonoid fractions from other parts of the plant, better antioxidant capacity and antimicrobial activity of these fractions from the tuberous root of *M. jalapa* have been reported.[4] Thus, it was observed that most studies were carried out using a crude extract of a particular solvent or a fraction to determine either various

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functional activities or the content of major compounds. In this investigation, we aimed to isolate and characterize bioactive components from *M. jalapa* radix.

2. Materials and methods

2.1. Chemicals

Thin-layer chromatography (TLC) silica gel 60 F_{254}, chemicals, and solvents of analytical grade were purchased from Merck, HiMedia, and Fisher Scientific (Mumbai, Maharastra, India).

2.2. Plant material

Mirabilis jalapa L. (紫茉莉 zǐ mò lì) was collected from locally grown flower gardens in Tezpur (91°48′E and 26°38′N), Assam. Voucher specimen was authenticated with the help of the Botanical Survey of India, Shillong (No. BSI/ESC/2011/Plant identification/93).

2.3. Extraction and fractionation

The tuberous roots collected were thoroughly washed with tap water and finally rinsed with distilled water. The roots were shade dried for 2 weeks and powdered. Approximately 50 g of ground sample was extracted with 500 mL of 80% methanol for 48 hours. Extraction was repeated and the extracts were pooled and filtered through Whatman number 1 filter paper. The filtrate was concentrated under reduced pressure in a rotary vacuum evaporator (RV10 Control, IKA, Germany). The concentrated extract was air dried to a constant weight (16.1% yield) at room temperature.

Approximately 10 g of aqueous methanolic extract of *M. jalapa* was dissolved in 100 mL of methanol, which was concentrated to one tenth of its volume, and acidified with 10 mL of 2M H_2SO_4. The acidified extract was extracted three times with chloroform. The pooled chloroform extract was named fraction 1 (F1). The remaining aqueous methanol solution was basified with NH_4OH and extracted three times with ethyl acetate. The ethyl acetate extract was named fraction 2 (F2). The remaining basified aqueous methanol was further extracted three times with isoamyl alcohol and this extract was named fraction 3 (F3). The remaining extract was termed as fraction 4 (F4).[4,5]

2.4. TLC studies of F1 and F3

Phytochemical screening indicated steroids and triterpenoids as the major compounds in F1, and flavonoids as the major compound in F3.[4] F1 and F3 of aqueous methanolic extract were subjected to TLC studies for isolation of potential free-radical scavenging and antimicrobial spots.

2.5. Preparation of TLC plates for collection of spots

Silica gel H without $CaSO_4$ was used as the adsorbent for collection of spots in TLC. The TLC plates were prepared and heated for activation in an oven for 30 minutes at 110°C. The precoated TLC plates were also used for observation of spots after collection.

Approximately 10 mg/mL of F1 and F3 in methanol and 5 mg/mL of standard solutions (β-sitosterol, betulinic acid, ursolic acid, oleanolic acid, rutin hydrate, quercetin, kaempferol, and chlorogenic acid) in methanol were prepared for spotting on the prepared TLC plates. The plates were spotted with 5–10 μL of samples

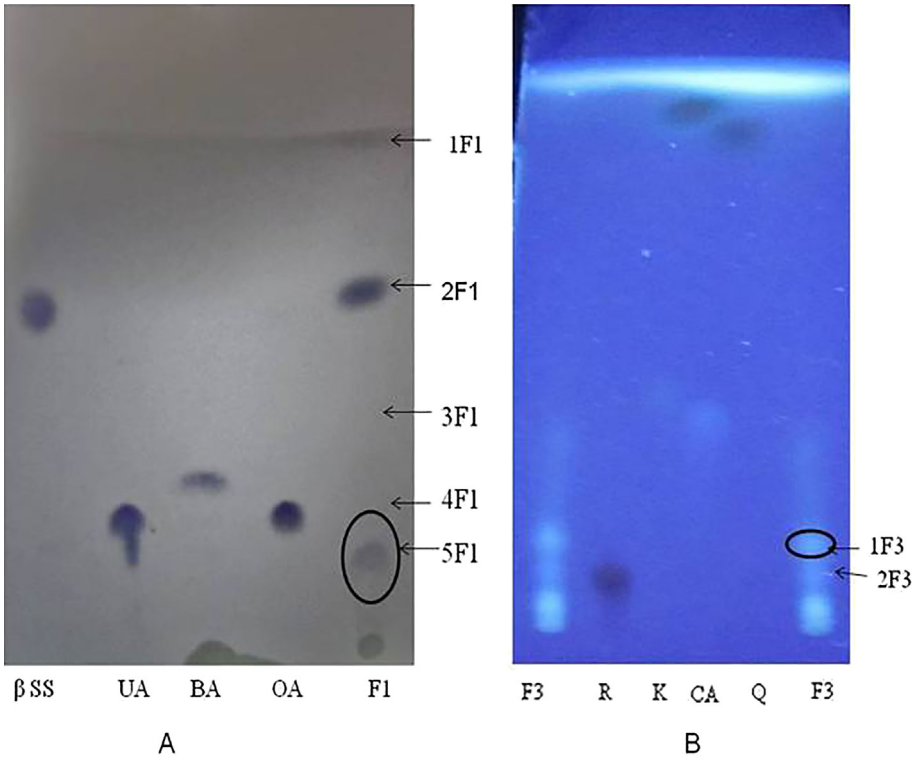

Fig. 1. Chromatograms of fractions F1 and F3. β-sitosterol (βSS), ursolic acid (UA), betulinic acid (BA), and oleanolic acid (OA) are the standards for detection of steroids and terpenoids in F1. Rutin (R), quercetin (Q), kaempferol (K), and chlorogenic acid (CA) are the standards for detection of flavonoids in F3. (A) Solvent system for separation of F1 [petroleum ether/chloroform/methanol (49:50:1)]. (B) Solvent system for separation of F3 [chloroform/ethyl acetate/formic acid (5:4:1)]. 3F1 and 4F1 were found to be low in quantity and were visible only when spotted using a larger quantity.