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Original Article

Phytochemicals and antioxidant evaluation of *Ficus racemosa* root bark

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ABSTRACT

Aim: Isolation and characterization of secondary metabolites from *Ficus racemosa* root bark and antioxidant activity of root bark and heartwood.
Methods: Isolation of compounds using column chromatography, identification by spectral (IR, NMR and mass) studies, antioxidant activity employing DPPH and FRAP methods.
Results: Compounds isolated include one long chain hydrocarbon (n-hexacosane), eight triterpenes (polypodatetraene, α-amyrin acetate, gluanol acetate, lupeol acetate, β-amyrin acetate, 24,25-dihydroparkeol acetate, α-amyrin octacosanoate including a novel lanostane derivative, lanost-20-en-3β-acetate), an isocoumarin (bergenin) and two phytosteroids (β-sitosterol and β-sitosterol-β-D-glucoside). Significant antioxidant activity was observed.
Conclusion: All compounds except β-sitosterol are being reported for the first time from the root bark of this species. Root heartwood was found to be a more effective antioxidant agent.

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1. Introduction

The Moraceae family commonly called Fig family is one of the most abundant and ecologically important family comprising of about 1000 species distributed in 40 genera.[1] *Ficus* is a large genus of woody trees, shrubs, vines and epiphytes widely distributed throughout the tropics of both hemispheres with about 850 species of which approximately 65 species are found in India.[2]

The species, *Ficus racemosa* Linn. syn. *F. glomerata* Roxb. (Vern. Gular) is large sized spreading tree commonly known as 'Cluster-fig' found throughout the greater part of India. The stem bark is antiseptic, antipyretic and used in the treatment of various skin diseases, ulcers, diabetes, piles, dysentery, asthma, gonorrhea, menorrhagia, leucorrhea, hemoptysis and urinary diseases. Fruits are a good remedy for visceral obstruction and also useful in regulating diarrhea and constipation.[3] A uterine tonic prepared using the aqueous extract of fruits was found to show effect similar to oxytocin.[4] Antiulcer, hypoglycemic and antioxidant activities from fruits have been reported.[5] Antioxidant, anti-inflammatory, antifungal, analgesic, antipyretic, antibacterial, antidiarrheal, hepatoprotective, hypotensive and various other activities of the leaves have also been evaluated.[6,7] A glance at literature revealed the isolation of triterpenoids, steroids, coumarins and phenolic esters from fruits, latex, leaves, heartwood and stem bark[5] and only one reference reporting the isolation of β-sitosterol from root bark.[8]

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Since the plant is medicinally important, therefore, the present work with the object to identify the secondary metabolites in the *F. racemosa* root bark and investigate the antioxidant capacity of root bark and heartwood was undertaken.

2. Material and methods

2.1. General

Melting points were recorded in open glass capillaries in Toshniwal apparatus. The IR spectra were recorded on a Shimadzu 8400S FTIR spectrometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded at 300 MHz and 75 MHz respectively on Jeol AL 300 MHz spectrometer using $CDCl_3$ and DMSO-d_6 as solvents and TMS as the internal reference. Mass spectra were recorded on Waters Xevo Q-TOF spectrometer. The fractionation was performed in Chromatographic column using silica gel 60–120 mesh (Merck) and thin layer chromatograms were conducted on Merck silica gel G plates. In general, spots were visualized under UV light as also spraying ceric ammonium sulfate followed by heating at 100 °C. The *in vitro* antioxidant activity experiments were monitored by UV–visible spectrophotometer (Pharmaspec-1700 Shimadzu).

2.2. Reagents

Silica gel 60–120 mesh (Merck) was used for column chromatography. Silica gel 60 F_{254} precoated aluminium sheets (0.2 mm, Merck) were employed for TLC. DPPH was purchased from Himedia while ascorbic acid, phosphate buffer, potassium ferrocyanide and trichloroacetic acid from Sigma Aldrich (India).

2.3. Plant material

The botanical material of *F. racemosa* Linn., Moraceae was collected from University of Rajasthan Campus, Jaipur, Rajasthan, India in March 2010 and authenticated by Herbarium of the Department of Botany, University of Rajasthan, Jaipur where a voucher specimen (No. RUBL 19764) is deposited.

2.4. Extraction and isolation of constituents

The root bark were shade dried (3.8 kg), powdered and exhaustively extracted with ethanol (95%) on a steam bath for 8 h thrice. The extract was concentrated under reduced pressure and left overnight at room temperature when a light brown solid deposited at the bottom of the flask. This ethanolic extract residue (4.5 g) was dried and the mother liquor on concentration *in vacuum* using rotary flash evaporator afforded a dark brown semi-solid (104.5 g) which was successively re-extracted with pet. ether (60–80%) followed by dichloromethane which on concentration afforded dark brown solids (2.4 g and 5.3 g respectively). Since the pet. ether and dichloromethane fractions exhibited a similar TLC profile (benzene:ethyl acetate, 1:1), they were mixed together for further studies. The ethanolic extract residue was chromatographed on an open normal silica column ($h \times Ø = 40 \times 2$ cm) eluted with pet. ether with increasing amount of EtOAc affording n-hexacosane (0.198 g), polypodatetraene (semisolid), α-amyrin acetate (0.159 g), gluanol acetate (0.356 g), lupeol acetate (0.216 g), β-amyrin acetate (0.198 g) and bergenin (0.251 g). The pet. ether and dichloromethane fractions on column chromatography yielded 24,25-dihydroparkeol acetate (0.224 g), lanost-22-en-3β-acetate (0.175 g), gluanol acetate (0.229 g), lupeol acetate (0.140 g), α-amyrin octacosanoate (0.162 g), β-sitosterol (0.128 g) and β-sitosterol-β-D-glucoside (0.056 g) (Fig. 1).

2.5. Antioxidant activity

2.5.1. DPPH radical scavenging activity

The DPPH radical scavenging activity was determined by the method of Fogliano et al.[9] A solution (2.5 ml) of 2×10^{-3} μg/ml of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with equal volume (2.5 ml) of extract/test compound/ascorbic acid (standard) at different concentrations (10, 20, 40, 60, 80 μg/ml) in methanol. The mixture was shaken vigorously, and then kept in dark for 30 min. The absorbance was monitored at 517 nm using UV–Vis spectrophotometer. Blank was also carried out to determine the absorbance of DPPH, before interacting with the sample.

The IC_{50} is the concentration of an antioxidant at which 50% inhibition of free radical activity is observed. The decoloration i.e. DPPH scavenging effect (% inhibition) was plotted against the sample extract concentration and a logarithmic regression curve was established in order to calculate the IC_{50}.

2.5.2. FRAP total reduction capability

$Fe^{3+} - Fe^{2+}$ transformation assay was carried out by Oyaizu's method.[10] To 1 ml of extract/test compound/ascorbic acid (standard) at different concentrations (62.5, 125, 250, 500, 1000 μg/ml) in ethanol was added 1 ml of distilled water, 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixture was incubated at 50 °C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added to the mixture, which was then centrifuged for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%) and the absorbance was measured at 700 nm using UV–Vis spectrophotometer. A greater absorbance value indicates greater reducing power.

2.6. Statistical analysis

The data are presented as mean ± standard deviation of three determinations. Statistical analyses were performed using a one-way analysis of variance. Results were calculated by employing the statistical software (SPSS). Data are expressed as mean ± standard deviation ($n = 3$). P values: $P < 0.05$ (a); $P < 0.01$ (b); $P < 0.001$ (c) compared to the control value, respectively.

3. Results and discussion

3.1. Identification of the isolated compounds

n-Hexacosane (1): mp 56–58 °C,[11] white solid, $C_{26}H_{54}$, *m/z* 366 (M^{+}), IR (υ_{max}) cm^{-1} (KBr): 2940, 2880, 730, 720.

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