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Extraction, isolation and identification of flavonoid from *Chenopodium album* aerial parts

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ABSTRACT

Chenopodium album L., (*C. album*) (family: Chenopodiaceae) is an annual shrub widely grown in Asia, Africa, Europe and North America. It is commonly known as Bathua (in Hindi), pigweed, fat hen or lamb-quarters. The leaves of *C. album* are applied as a poultice to bug bites, sunstroke, rheumatic joints and as mild laxative. The flavonoids contained in *C. album* aerial parts were extracted, identified and characterized. Sequential soxhlet extraction was subjected to preliminary phytochemical screening and flavonoid quantification. The results showed that maximum yield of the flavonoid (7.335 mg/g) were obtained from acetone extract. This acetone extract was subjected to flash chromatography for isolation of flavonoid. Characterization of isolated flavonoid was done by UV, IR, 1H & 13C NMR and MS. On the basis of chemical and spectral analysis structure was elucidated as 2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one, a flavonoid.

1. Introduction

Polyphenols appear to be important metabolic modulators by virtue of their ability to influence several cellular pathways and molecules that have been reported as potential targets for polyphenolic compounds. Flavonoids are “the most common group of polyphenolic compounds in the human diet and are found *ubiquitously* in plants”. Although more than 4000 flavonoids have been identified, several appear to be important components of many fruits and vegetables. According to the differences in functional groups and their relative positions of the 15-carbon skeleton (aglycons), flavonoids are classified into several subgroups such as flavone, flavanone, flavonol, isoflavonoid, anthocyanidin, and chalcones.[1] Flavonols, the original bioflavonoids such as quercetin, are also found ubiquitously, but in lesser quantities. Flavonoids, a subclass of polyphenols, are a group of phytochemicals that are among the most potent and abundant antioxidants in our diet and also possesses various activities such as anti inflammatory, anti cancer etc.

Chenopodium album L., (*C. album*) (family: Chenopodiaceae) is an annual shrub widely grown in Asia, Africa, Europe and North America. It is commonly known as Bathua (in Hindi), pigweed, fat hen or lamb-quarters. The leaves of *C. album* are applied as a poultice to bug bites, sunstroke, rheumatic joints and as mild laxative.

The plant is used in folk medicine in different parts of the world as diuretic, laxative, sedative, hepato-protective and antiparasitic. The leaves possesses anthelmentic, antiphlogistic, antirheumatic, mildly laxative and odontalgic properties, applied as wash or poultice to bug bites, sunstroke, rheumatic joints and swollen feets.[2] Additionally, decoction of its aerial parts mixed with alcohol was used in the rheumatism.[3] Cinnamic acids amides.,[4] flavonoids[5,6] and apocarotenoids[7] have also been isolated from this species. Flavonoid from *C. album* has significant potential to scavenge free radicals, NF kappa B inhibition, anti-inflammatory, and hence have got antirheumatic potential.[8]

The aim of the study was to isolate the flavonoids using Flash Chromatography and was characterized through their spectral analysis like IR, 1H, 13C NMR and MS.

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2. Material and methods

2.1. Chemicals and standard drugs

All the chemicals and solvents used were of analytical grade, Silica gel (G) 60 F and 0.25 readymade aluminum sheets (Merck, Germany), Rutin and Quercetin from SD fine Chem. Ltd. Mumbai.

2.2. Plant material and preparation of extract

The aerial parts were collected from Ramtek region in the month of August, authenticated by Dr. (Mrs.) Alka Chaturvedi, Department of Botany, R.T.M. Nagpur University, Nagpur. A voucher specimen has been deposited in the Herbarium of Department of Botany, with collection number RA 9576.

The aerial parts of *C.album* were dried under shade and pulverized to a coarse powder. The powdered crude material (1 kg) was defatted with petroleum ether and then extracted successively with ethyl acetate, acetone and methanol using Soxhlet extractor followed by cold maceration (7 days) with 50 % methanol. The extracts were concentrated using rotary vacuum evaporator to yield ethyl acetate extract (EACA; yield: 3.9 % w/w), acetone extract (ACCA; yield: 4.79 % w/w), methanolic extract (MECA; yield: 13.58 % w/w) and 50 % methanolic extract (HACA; yield: 12.76 % w/w). These extracts were subsequently subjected to phytochemical screening and quantitative estimation.

2.3. Phytochemical screening[1,9]

The extracts were screened for the presence of different phytochemicals employing thin layer chromatographic (TLC) techniques. Thin layer plates precoated with silica gel G (Merck, 0.25 mm thickness) were used. Development was carried out with different solvent systems such as ethyl acetate: methanol: water (100:13.5:10, v/v/v), ethyl acetate: formic acid: acetic acid: water (100:11:11:26, v/v/v/v), chloroform: methanol: water (70:30:4, v/v/v), toluene: ethyl acetate: diethylamine (70:20:10, v/v/v) and ethyl acetate: methanol: water: acetic acid (65:15:15:10, v/v/v/v). After development of chromatogram in the solvents, the plates were dried and sprayed with $AlCl_3$ reagents for the detection of flavonoids. Visualization was carried out under visible and UV light (λ: 366 nm). The quantitative estimation of flavonoid in EACA, ACCA, MECA and HACA extracts were carried out respectively.

2.4. Determination of flavonoids (TFA)

Flavonoid content was determined by the aluminium chloride method.[10] Briefly, to 1 ml of test solution (1 mg/ml), 1.5 ml of 95 % alcohol, 0.1 ml of 10 % aluminum chloride hexahydrate ($AlCl_3.6H_2O$), 0.1 ml of 1 M sodium acetate (CH_3COONa) and 2.3 ml of distilled water was added. After incubation at room temperature for 40 min, absorbance of the reaction mixture was measured at 435 nm against corresponding blank, prepared in the same manner without adding $AlCl_3$. Rutin was used as a reference standard and results were expressed as mg of rutin equivalents (RE)/g of extract. All determinations were performed in triplicate.

2.5. Isolation using flash chromatography[11]

Flash column chromatography was performed on spherical silica gel, C-18; 40–63 micron (230–400 mesh), 60Å pore size, pH range of 6.5–7.5, in glass columns designed for FCC. Sorbent Technologies (Atlanta, GA, USA) supplied silica gel. Glass flash columns were manually dry packed using C-18 silica. After the column was packed and mounted on the instrument, a volume of initial solvent mixture was pushed through the silica to remove air and to wet and equilibrate the column. The sample (Extract or fractions) previously triturated with three times column silica and dried was placed in between Teflon disc in solid sample loading cartridge. Sample loaded cartridge was placed over head of the column before elution. Fractions were collected in test tubes, graduated with the fraction volume and number. The various flash chromatographic conditions used for the separation of compounds were mentioned in Table 1.

2.6. Compound characterization

For characterization of compound UV, IR, NMR and Mass spectra were performed. The UV absorption of isolated constituent was determined in methanol over a scanning range of 200–800 nm. The compound was dissolved in methanol to obtain required concentration and spectrum was recorded. The infrared absorption spectrum of the isolated constituent as KBr disc has been determined on FTIR-8101A (Shimadzu) and the absorption peaks in the form of wave numbers (cm^{-1}) were noted.

1H NMR spectra were acquired on a Bruker DSX-300 spectrometer, using the standard pulse program 'lc1pnf2', which is based on the one dimension version of the NOESY sequence and allows double pre-saturation, to suppress the water peaks. 32k data points were recorded over a sweep width of 9191 Hz, with 512 scans. An exponential line broadening of 1 Hz was imposed on the accumulated data before Fourier transformation. The 13C NMR experiments were obtained at 400.23 MHz on a Bruker Biospin Ultrashield plus AV-400 MHz instrument. The samples were dissolved in deuterated methanol. The mass spectroscopy of isolated constituent was carried out using a TOF MS ES + mass spectrometer and experiments were acquired using the potential LIFT technique based on post source and post-decay acceleration of fragment ions. MS spectra were annotated using Flex Analysis software and transferred to BioTools for sequence evaluation. The spectrometer was operated in reflection mode optimized for positive ions with masses from 0 to 2000 Da with 25 kV acceleration voltage. The nitrogen laser extinction frequency was set at 10 Hz. The laser power was optimized to obtain a good signal-to-noise ratio after averaging 100 signal-shot spectra. Mass spectra were acquired at National Institute of Pharmaceutical Education & Research, Kolkata, India.

Table 1
Method parameters of flash chromatographic separation of ACCA extract.

Instrumentation	Teledyne Isco CombiFlash® Companion™ 4x	
Column	24 g C-18 Reversed Phase RediSep Silica	
Run length	270 min	
Flow Rate	21 ml/min	
Equilibration Volume	100.0 ml	
Peak Detection	Slope-based Sensitivity: High **Peak Width**: 1 min or **Thresholding**: 0.05AU	
Detection wavelength (red)	254 nm	
Monitor wavelength (purple)	267 nm	
Mobile phase	**Solvent A**: mixture of 0.1% formic acid in water (v/v) **Solvent B**: Acetonitrile	
Gradient[a]	**% of Solvent B**	**Column Vol. (CV)**
	5%	3
	a linear gradient to 80%	35
	a linear gradient to 100%	50
	100%	55
	Ramp back to 5% B	55–60
	5%	60–65

[a] Mobile phase composition was freeze during the peak elution and continued with the gradient elution after peak retaining base line.

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