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Isolation and characterization of flavonoid from *Alternanthera sessilis*

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Abstract

In the present work, the primary objective was to isolate flavonoid from the hydro-alcoholic extract of *Alternanthera sissilis* plant. The plant was powdered after drying and extracted with ethanol-water (70:30) after defatting with petroleum ether, to obtain dark brown sticky extract in 16.37% yield. The qualitative phytochemical screening suggested the presence of alkaloid, glyocoside, tannins, phenolics, flavonois and sterols in the extract. The amount of phenolic present in the hydro-alcoholic extract was found to be 109.18 GAE mg/g of extract.The amount of phenolic present in the hydro-alcoholic extract was found to be 76.27 QE mg/g of extract. The amount of phenolic present in the hydro-alcoholic extract was found to be 50.16 CE mg/g of extract. The hydro-alcoholic extract was analyzed by TLC for separation of components. The optimized solvent system was Toluene: Ethyl acetate: Acetic acid (4:1:0.2) and resulted in 6 components from the hydro-alcoholic extract. Active constituents were isolated from column chromatography with the mobile phase of Toluene: Ethyl acetate: Acetic acid (4:1:0.2) to obtain 18 fraction of 25 mL each. The fraction F3 was found to contain a single compound as visualized from TLC and was evaporated to obtain the solid product. This solid was recrystallized from propan-2-ol to obtain the pure isolated compound. The isolated compound was studied using FT-IR, mass and NMR spectral characterization to identify the compound. The isolated compound was proposed to have flavonoid structure and from the molecular weight it was proposed to be the flavonoid quercetin-O-glucoside.

Introduction

Flavonoids are an important class of natural products; particularly, they belong to a class of plant secondary metabolites having a polyphenolic structure, widely found in fruits, vegetables and certain beverages. They have miscellaneous favourable biochemical and antioxidant effects associated with various diseases such as cancer, Alzheimer's disease (AD), atherosclerosis, etc.[1-3] Extensive biological studies of flavonoids have revealed their health benefits including disease prevention.[4] They have exhibited anti-oxidant, anti-inflammatory, anti-bacterial, and anti-viral activities, anti-oxidant, anti-allergic anti-carcinogenic properties. The protective effects of flavonoids have been reported as they help to reduce oxidative stress in the body. The cholesterol-lowering activity, anti-cancer, anti-oxidant of myricetin, tricin, apigenin, luteolin, quercetin, and isorhamnetin has been reported.[5] The anti-viral, anti-bacterial, anti-cancer, cardioprotective, and anti-inflammatory activity, as chelating agents and, are strong topoisomerase inhibitors anti-aggregational, anti-atherosclerotic, and detoxification activities of various flavonoids have been reported.[6] These biological activities depend to a larger extent on the hydroxyl group in the flavonoids. Quercetin has reduced the risk of cancer, eye diseases, arthritis, and allergic disorder. The decreased risk of cardiovascular disease by proanthocyanins and flavone-3-ols has been reported. *Althernanthra sessilis* is a plant of the Amaranthaceae family. Literature has shown that *Alternanthera sessilis* is a good source of several phenolic compounds, though not much flavonoid components have been isolated from the plant.[7] Also the extracts of the plant are also known to exhibit several pharmacological actions.[8] Considering the above factors it was envisioned to isolate and characterize flavonoid from hydroalcoholic extract of *Alternanthera sessilis*.

Material and Methods

Collection and preparation of plant material

The whole plant of *Alternanthera sissilis* were collected from the local surrounding of Bhopal, Madhya Pradesh in the month of January, early in the morning and authenticated by botanist at RB Science, Bhopal. The collected plant, after authentication were washed with distilled water and dried under shade. The completely dried leaves were converted to fine powdered form with the aid of a blender at low speed. The powdered leaves were stored in air tight container and marked appropriately till taken for further processes and investigation.

Extraction of plant material[9]

The plant powder prepared using the above procedure were used for extraction process. Hot continuous extraction was performed for extracting out the phytochemicals from the plant powder. Briefly, 98 g of the plant powder was evenly packed in the extractor of the soxhlet apparatus and extracted successively with various solvents of increasing polarity. The solvents used for extraction included petroleum ether (500 mL), and ethanol-water (70:30) (500 mL). The extraction process was carried out for about 7-8 h for each solvent, till a clear solution was visible in the siphon tube of the extractor. The extracts (solvents) were filtered while hot to remove any un-dissolved material (debris or impurities). The extracts were concentrated by evaporation using rotary vacuum evaporator to obtain viscous liquid. The concentrated extracts were then transferred to 100 mL beaker and the remaining solvents were evaporated on thermostatically heated water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture. The dried extracts were stored in desiccators until used for further investigational procedures.

Preliminary phytochemical screening of extracts[10,11]

The hydroalcoholic extract was subjected to qualitative phytochemical testing procedures for identifying the presence or absence of usual plant secondary metabolites. The test was performed for alkaloids, triterpenes/steroids, glycosides, tannins, flavonoids, saponins, and phenolic acids. The color, intensity of color or the precipitate formation was used as observational responses to the reactions occurring in these tests.

Total Phenolic Content Determination

A small quantity of extract (0.1g) was mixed with 8 mL of methanol and kept overnight. The suspension was filtered through a qualitative cellulose filter paper and the filtrate was diluted to 10 mL with methanol. The solution was stored at 4°C in amber color bottles and served as the stock solution (50 mg/mL) for subsequent analyses. In order to determine the total phenolic content, 200 μL of the extracted sample was mixed with 1.4 mL purified water and 100 μL of Folin-Ciocalteu reagent. After at least 30 s (but not exceeding 8 min), 300 μL of 20%Na_2CO_3 aqueous solution was added and the mixture was allowed to stand for 2 h.[12,13] The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the calibration curve. The control solution contained 200 μL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Total Flavonoid Content Determination

Determination of total flavonoids content was based on aluminium chloride method. 50 mg quercetin was dissolved in 50 ml methanol, and various aliquots of 25- 150μg/ml were prepared in methanol. 0.1 g of dried extract was extracted with 10 ml methanol, filtered, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of flavonoid. 1 ml of 2% $AlCl_3$ methanolic solution was added to 1 ml of extract or standard and allowed to stand for 60 min at room temperature; absorbance was measured at 420 nm.[14]

Total Tannin Content Determination

The tannin contents or Proanthocyanidin were determined by method of Broadhurst et al., 1978 with slight modification, using catechin as a reference compound. A volume of 400μL of extract is added to 3 mL of a solution of vanillin (4% in methanol) and 1.5 mL of concentrated hydrochloric acid. After 15 min of incubation the absorbance was read at 500 nm. The condensed tannin was expressed as gE.Catechin/100g.[15]

Preliminary Thin layer chromatography

Thin layer was done on precoated silica gel TLC plates. The mobile phase for TLC was optimized to obtain the maximum number of components separated from the extract. Once the chromatogram was developed, the R_f Value of the spot was calculated using the formula.

$$Rf\ value = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

Column chromatography of extract

Active constitutes were isolated from extract by column chromatography. Solvent system was developed in preliminary TLC in which the maximum spots were visible. The developed solvent system comprised of Toluene: Ethyl acetate: Acetic acid (4:1:0.2). The same solvent system was utilized as the mobile phase for column chromatographic isolation of phyto molecule.

The column was packed with the slurry of silica gel (60-120 mesh) in the mobile phase. The solvent was then allowed to drain out allowing the formation of a uniform separation bed of the stationary phase. The sample was loaded onto the top of the separation bed with the help of Pasteur pipette and the eluting solvent (mobile phase) was added. Gravity pulled the mobile phase down through the stationary phase and the components in the mixture move through the column at different rates based on their polarity. Fractions of 20 mL were collected in Erlenmeyer flasks and the TLC of each flask content was performed. The fractions with similar TLC spots were pooled together and the solvent was removed by evaporation and the pure components can be further analyzed.[16]

Spectroscopic characterization of the isolated products

The characterization of the isolated product was performed by UV, NMR, FT-IR and Mass spectral studies.

Results and Discussion

Extraction and phytochemical screening

The hydro-alcoholic extract after defatting with petroleum ether was found to be sticky dark brown color with a yield of 16.37%. The qualitative phytochemical screening of the extracts was done by various test either directly employing the test reagent or by addition of various reagents leading to formation of precipitate or color change. The extract revealed the presence of alkaloids, glycosides, phenolics, flavonoids, tannins, sterols and terpenoids.

Total phenolic, flavonoid and tannin content

The amount of phenolic present in the hydro-alcoholic extract was found to be 109.18 GAE mg/g of extract. The amount of phenolic present in the hydro-alcoholic extract was found to be 76.27 QE mg/g of extract. The amount of phenolic present in the hydro-alcoholic extract was found to be 50.16 CE mg/g of extract.

TLC of extract

The hydro-alcoholic extract was analyzed by TLC for separation of components. The optimized solvent system was Toluene: Ethyl acetate: Acetic acid (4:1:0.2) and resulted in 6 components from the hydro-alcoholic extract (Figure 1). The standard quercetin and the petroleum ether extract were also spotted on the same TLC plate. No separation was visible in the petroleum ether extract. Migration of component to retention factor equivalent to quercetin was visible in the hydro-alcoholic extract.

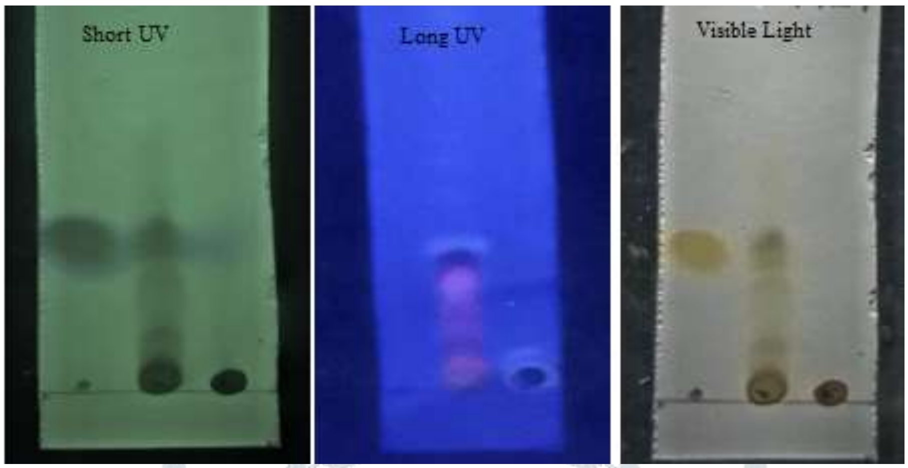

Figure 1 TLC of the crude extract and quercetin

Isolation of flavonoid

Active constitutes were isolated from column chromatography with the mobile phase of Toluene: Ethyl acetate: Acetic acid (4:1:0.2) to obtain 18 fraction of 25 mL each. The fractions with same TLC profile were pooled to obtain 6 fractions F1 (1-3), F2 (4-5), F3 (6-10), F4 (11-13), F5 (14-15) and F6 (16-18). The fraction F3 was found to contain a single compound as visualized from TLC and was evaporated to obtain the solid product. This solid was recrystallized from propan-2-ol to obtain the pure isolated compound. The isolated compound was studied using FT-IR, mass and NMR spectral characterization to identify the compound.

The UV spectra of the isolated compound exhibited absorption maximum at 256.0 nm (Figure 2).

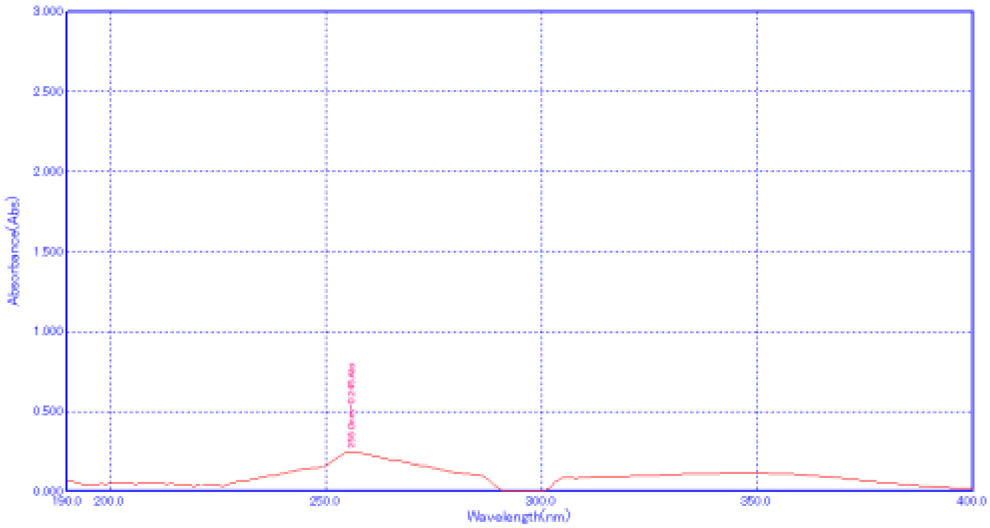

Figure 2 UV spectra of isolated compound

The FT-IR spectra (Figure 3) revealed presence of stretching vibration of functional groups corresponding to hydroxyl (3636.94 cm^{-1}), carbonyl (1723.47 cm^{-1}), conjugated alkene (aromatic) (1509.36-1601.95 cm^{-1}), alkene C-H (3054.41 cm^{-1}) and lactone (1781.34 cm^{-1}).

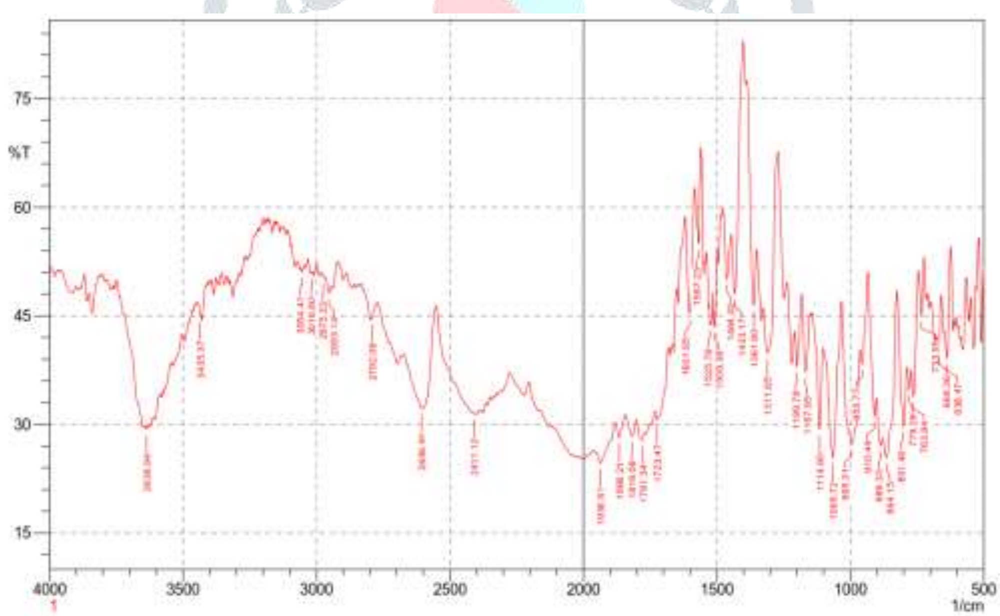

Figure 3 FTIR spectra of isolated compound

The UV and IR spectra were indicating to the presence of flavonoid in the isolated compound. The proton NMR spectra of the compound revealed the chemical shifts at 2.48-2.90 (OH aliphatic), 4.44-4.67 (CH2, methylene), 3.33-3.67 (CH, cyclic), 5.27-5.84 (OH, Aromatic) and 5.88-8.45 (CH, Aromatic) (Figure 4).

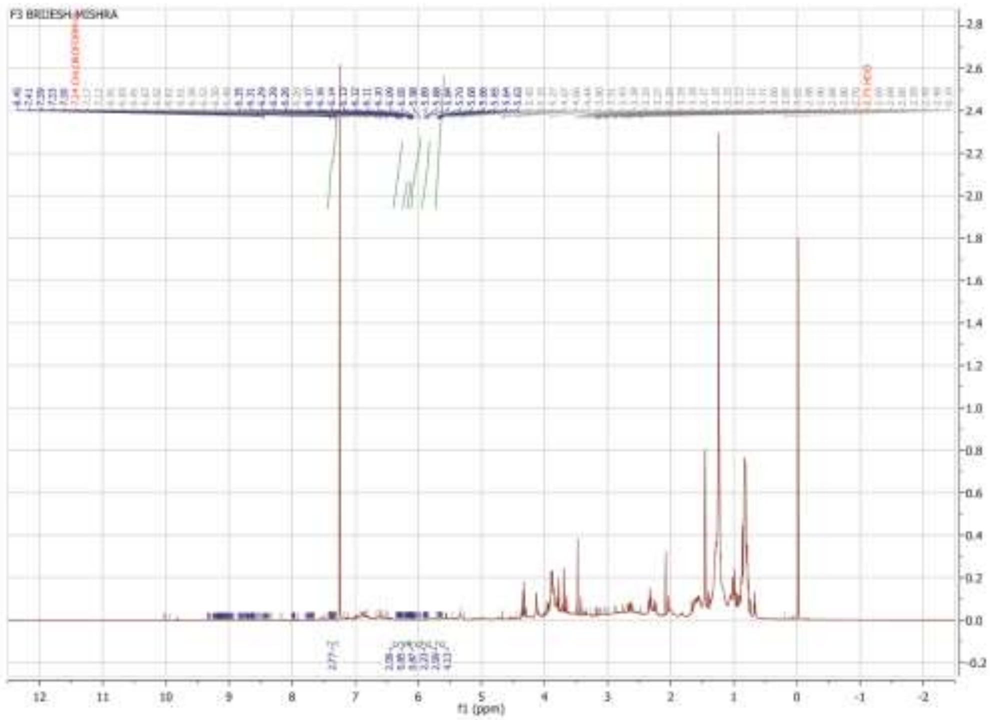

Figure 4 **[1]H NMR spectra of isolated compound**

The molecular weight of the isolated compound was interpret using the mass spectral data which revealed molecular ion peak at m/z value of 463.13. The base peak occurred at m/z value of 302.12 (Figure 5). The occurrence of the fragment peak could be attributed to the flavonoid quercetin whereas the molecular ion peak could be attributed to the compound as quercetin-O-glucoside.

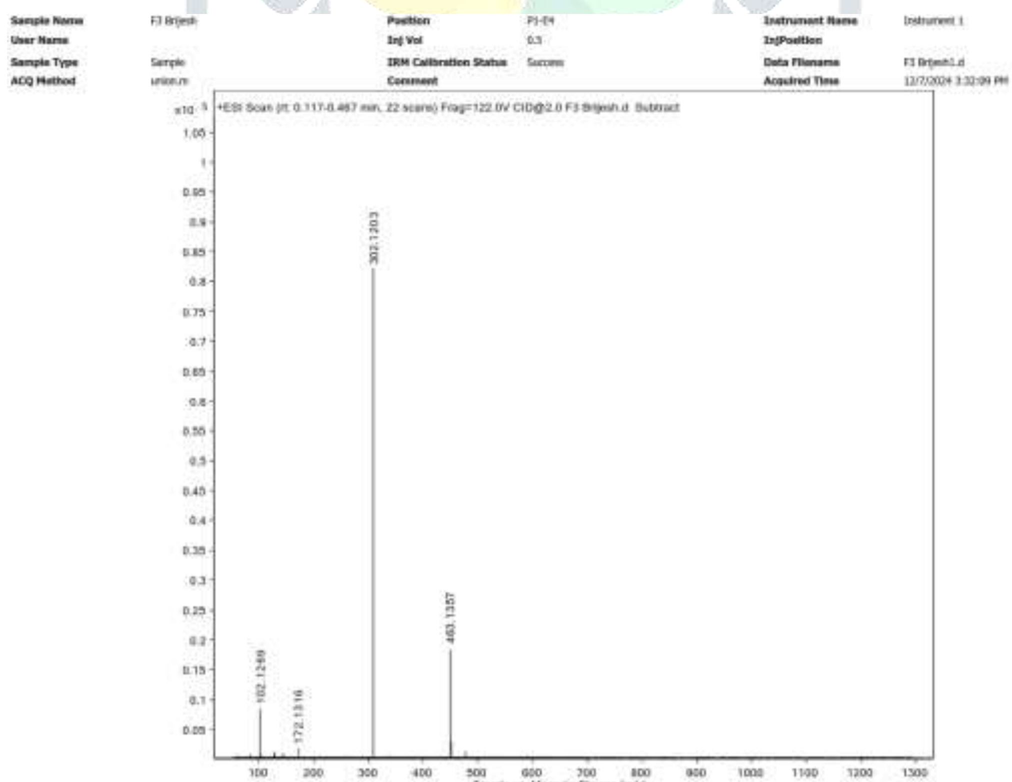

Figure 5 **Mass spectra of isolated compound**

The proposed structure of the isolated flavonoid quercetin-O-glucoside is presented in Figure 6.

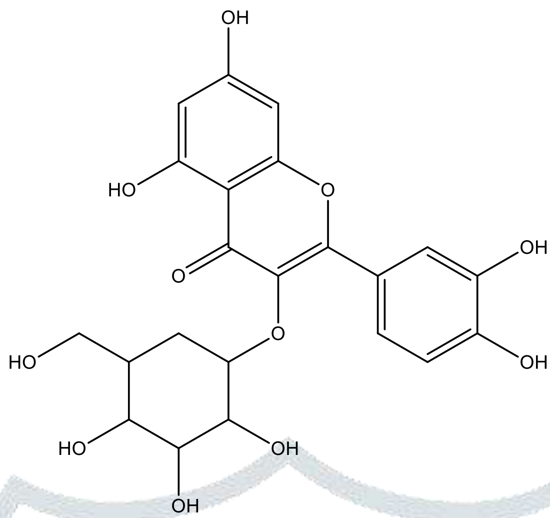

Figure 6 Proposed structure of isolated flavonoid

Conclusion

In the present investigation, a weed, *Alternanthera sissilis* used for its nutritive value was extracted with a blend of ethanol and water and flavonoid was isolated from the extract by column chromatography. The isolated compound was found to contain fragment peak of quercetin in the mass spectra suggesting the isolation of quercetin derivative from the weed. The isolation of the flavonoid from the plant led us to conclude that along with nutritive potential, the plant is also a source of anti-oxidant compounds which would be beneficial in using the plant for medicinal purposes in further studies.

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