

Phytochemical and Chromatographic studies in the flowers of *Woodfordia fruticosa* (L) Kurz.

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ABSTRACT

*The present study primarily aims to carry out a preliminary phytochemical screening so as to detect the major class of compounds present. TLC profiling of the *Woodfordia fruticosa* flowers was carried out using sequential extracts of solvents with varying polarity; petroleum ether, chloroform and methanol respectively. The TLC documentation was done in short UV(254nm), long UV(365nm) and visible light after derivatisation with Anisaldehyde Sulphuric acid as the spray reagent.. Then DPPH free radical scavenging assay was carried out in the flowers so as to detect its antioxidant activity. The flower has a significant sweetness when tasted; so the total estimation for the starch content in the plant was carried out. The HPLC studies were performed in the methanolic extract of the plant, since it gave better separation than the other two solvents. The results obtained can be used for the genuine identification of the plant from its adulterants.*

Keywords: *Woodfordia fruticosa*, HPLC, DPPH.

INTRODUCTION

Traditional medicines have been used for many centuries by a substantial proportion of the population of India[1].The World Health Organization (WHO) estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs[2],[3].*Woodfordia fruticosa* is an important traditional medicinal plant belonging to the family Lythraceae. It is extensively used in the preparation of “Ahavas and Arishtas” containing self generated alcohol.The flowers of *Woodfordia fruticosa* Kurz. are commonly used for the treatment of several ailments which includes rheumatism, leucorrhoea, menorrhagia, asthma, liver disorder, and inflammatory conditions[4].The flowers are astringent, acrid, refrigerant, stimulant, depurative, typtic, uterine sedative, antihelminthic, constipating, antibacterial, vulnerary, corrective of apigments, alexeteric and febrifuge. It is also has

antibacterial[5], antifertility[6] activities. It's also used as a mordant in the preparation of dyes [7].

The three dimeric hydrolysable tannins woodfordins A, B, C isolated from dried flowers and their structures were established. Oenothin-b also obtained. Quercetin-3-O- α -L-arabinoside quercetin-3-O-6''- β -D-galactopyranoside and myricetin-3-O-arabinopyranoside were isolated from leaves. Woodfordin D and Oenothin A were isolated from dried flowers. Woodfordins E, F, G, H and I were also isolated and structures were elucidated. Malvidin, pentose, glycosides, quercetin, kaempferol-3-glycoside, hecogenin, carotene, carbohydrates, insulin, 3 mannitol, lawsone, aspartic acid, protein, riboflavin, citric acid, punicaline, estrone etc were also isolated [8].

In the present study preliminary screening, TLC profiling and HPLC analysis was done for *W. fruticosa* flowers. Estimation of total starch was also done in the extract. Authentication and standardization of the herbs has been the major problem in the natural product industry, this could up to an extent be limited by this methods.

MATERIALS AND METHODS

Plant Collection

The dried flowers of *Woodfordia fruticosa* were collected from local market, Calicut in Dec 2009 and authenticated from Karpagam College, Coimbatore. Voucher specimen is preserved in the herbarium of College. The plant material was then shade dried and powdered.

Preliminary Phytochemical screening

Methanolic extract in the concentration 100mgml⁻¹ was used for the detection of major class of chemicals like carbohydrates, phenols, flavonoids, tannins, alkaloids, glycosides, saponins, anthraquinones and amino acids present in the flowers.

Thin Layer Chromatography

About 10g of the dried plant material was extracted by refluxing sequentially with petroleum ether, chloroform and methanol for 6 hours with each of the solvents. Each extract was collected separately and was concentrated up to 10ml using a rotary flash evaporator. Pre-coated Merck silica gel plates 60 F 254 with layer thickness 0.25mm (E. Merck), micro syringe (25ml, Hamilton) (802) and a UV chamber. Concentrated plant extracts were applied to a TLC plate as 1cm bands. The plates were developed over 7.5 cm. Solvent system used was toluene: chloroform: methanol (8:2:1) for all the three extracts. The chromatograms were evaluated directly under UV_{254 nm} and UV_{365 nm} after staining with anisaldehyde sulphuric acid spray (ANS) reagent. Poured the reagent over the plate and the plate was thereafter heated for 5-10 min at 100°C within a hot air oven, and was then observed and documented. Lupeol standard marker was identified using TLC identity test [9].

Free Radical Scavenging Assay (DPPH)

About 5g of the dried plant material was taken and kept for extraction for 9 hours with methanol. The sample was collected in a beaker and then kept for concentrating over a waterbath.

Method

The concentrated sample was made upto 250ml in a standard flask using methanol. Antioxidant activity of *Woodfordia fruticosa* was measured by decreasing the absorbance of a methanolic solution of coloured DPPH brought about by the sample at 550 nm. A stock solution of DPPH (1.3mg/ml methanol) was prepared such that 0.3 ml of it in 10 ml methanol gave an initial absorbance of 0.8. This stock solution was used to measure the antioxidant activity. Decrease in the absorbance in the presence of methanolic extract of *Woodfordia fruticosa* at different concentrations was noted after 30 minutes. EC_{50} was calculated from percentage inhibition. Efficient concentration (EC_{50}) is the concentration of the substrate that caused 50% loss of DPPH activity BHA was used as the positive control. The Shimadzu UV-1700 model with a double beam spectrophotometer, Quartz cuvettes, methanol GR (Merck), Butylated Hydroxy Anisole (BHA) (Sigma), Diphenyl Picryl Hydroxyl (DPPH) (Sigma Aldrich) were used [10][11].

Estimation of Total Sugar in the Plant

About 1g of the plant material was homogenized with 50ml 80% ethanol, which was placed in a magnetic stirrer to remove sugars. The sample was centrifuged at 6000rpm for 15 mins and the supernatant was collected. From this 0.2 ml supernatant was pipetted out. The volume was made upto 1ml with water. 1ml of 5% phenol and 5ml of 96% H_2SO_4 was added and mixed well. It was placed in a waterbath 25-30°C for 20 minutes. The colour was read at 490nm. Total carbohydrate present in the sample solution was calculated by using 100mg of glucose standard in 100ml of water. 10ml of this solution was diluted to 100ml with distilled water and used as working standard. The standard graph was prepared with 0.2, 0.4, 0.6, 0.8 and 1.0ml of working standard taken in a series of the test tubes

High Pressure Liquid Chromatography (HPLC)

Instrumentation

The high-pressure liquid chromatographic system consists of Shimadzu LC – 10ATVP pump, a valve type injector, Shimadzu SPDM10 AVP model Photo Diode detector (Shimadzu, Tokyo, Japan), Phenomenex Luna C 18 (250×4.6 nm) column with a particle size of 5 μ . Acetonitrile, methanol (HPLC grade, E. Merck, India) in the ratio 5:5 were used for the analysis.

RESULTS

Preliminary screening

Preliminary screening of the flowers of *Woodfordia fruticosa* revealed the presence of carbohydrates, tannins and glycosides in major quantities, phenols in moderate quantities and anthraquinones and flavonoids in minor quantities.

Table 1

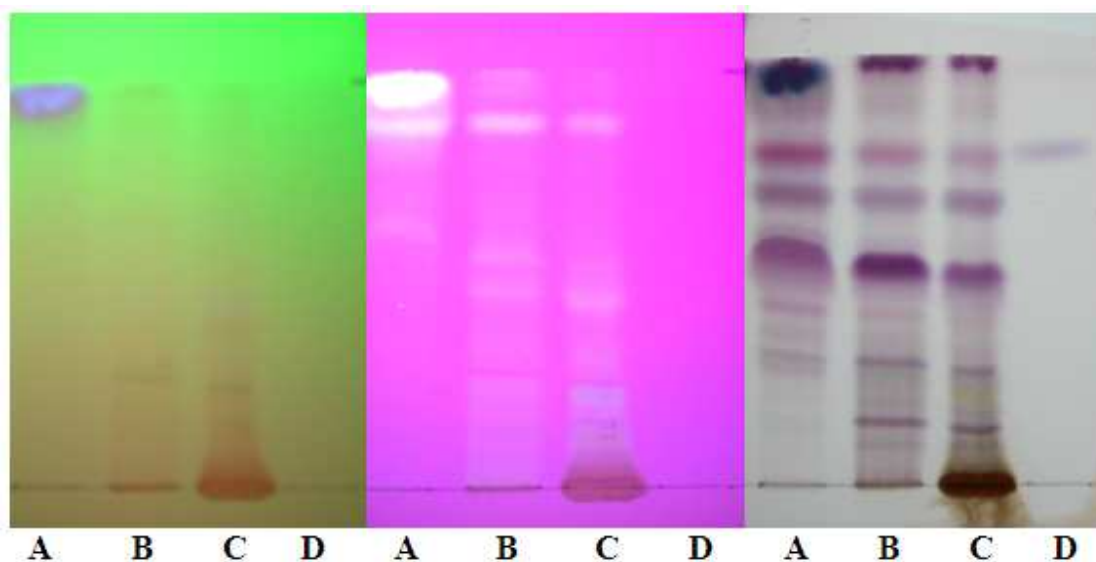
| Class | Carbohydrates | Phenols | Flavonoids | Tannins | Alkaloids | Glycosides | Anthraquinones | Amino acids |
|--------|---------------|---------|------------|---------|-----------|------------|----------------|-------------|
| Result | +++ | ++ | + | +++ | --- | +++ | + | --- |

+++ Major, ++ Moderate, + Traces, - Absence

Thin Layer Chromatography

TLC profiles and fingerprints were developed for each of the three extracts using hexane chloroform and methanol in the ratio 8:2:1 (**Figure 1**) and the retention factors for the separated compounds were calculated. (**Figure 2**). Best separation was obtained in methanolic extract.

Figure 1



A: Petroleum Ether B: Chloroform C: Methanol D: Standard Lupeol Marker

The concentration of the *Woodfordia fruticosa* FLOWERS was found to be 8mg/ml.

Graph 1: In-vitro antioxidant Assay (DPPH Assay)

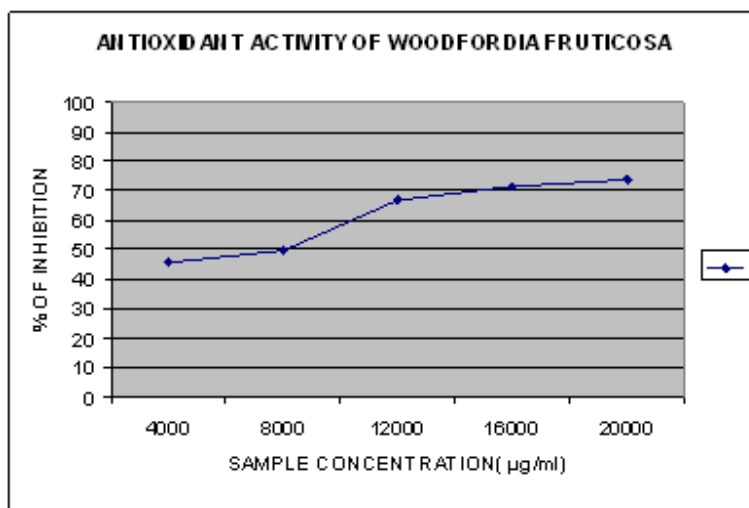
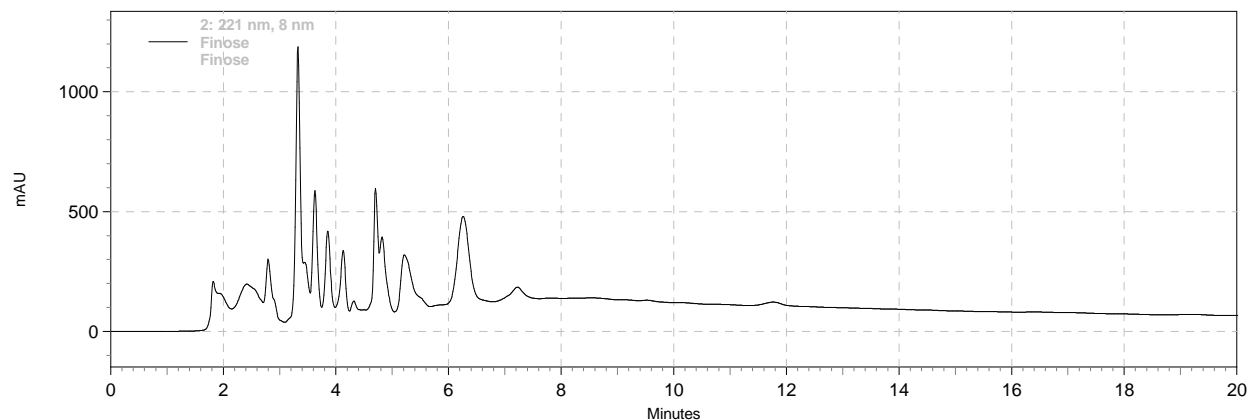


Figure 2: HPLC profile of the methanolic extract of *W.fruticosa*

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