Phytochemical studies of *Brenia retusa*

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Abstract

Plants are one of the primary sources of medicines since ancient times. *Brenia retusa* possesses significant amount of phytochemicals which can be used against various types of diseases. The richness of phenolics in *Brenia retusa*, shows the antioxidant potential of the plant.

Keywords: *Brenia retusa*, Phenolics, HPLC, Paper Chromatography

Introduction

Over the centuries an inseparable bonds are developed between human beings and plants because of their basic needs. The availability of novel molecules from current medicinal system get exhausted and gradually became resistance towards many diseases [1], [2]. In search of new potent molecules progress has been undergone towards medicinal plants [3].

Natural phenolics are one of the major subclass of secondary plant metabolites and also serve as dietary antioxidants. Phenolics play a major preventive role against many chronic diseases, such as lung cancer, asthma and cardiovascular diseases, found in several epidemiological investigations [4]. Many scientists shows the presence of phenolics and polyphenols in large numbers of plants as well as their plant products for examples fruits, vegetables, beverages, pulses and cereals etc.

Breynia retusa is a shrub having wide space extended branches. It found abundantly in many countries namely India, Sri Lanka, Nepal, Bangladesh, Bhutan, Cambodia, Thailand, China, Laos, Malaysia, and Vietnam [5].

These shrubs are approximately 1-3.5 m tall, erect, slender branches with the leaves 1.25-2.5 cm in size, distichous, broadly elliptic glabrous throughout. 1-2.5 mm petiole are present with blade elliptic to slightly obovate leaves 1-2 in numbers are present, papery or thinly papery, abaxially grayish green or pruinose, adaxially green, base obtuse to rounded, apex rounded to subacute, mucronulate; lateral veins 3-7 pairs, slender, obscure. Small stipules 1-2 in numbers are present with 0.6-0.7 mm. Flowers are small in size, solitary or axillary on filiform pedicels, the males in the lower and females in the upper axils. Male flowers pale yellow, with slender pedicels. calyx is 2-3 mm in diameter having campanulate to turbinate shape with 6-lobed; sepals rounded to oblong; 3 stamens are present with connate into 3-angled head, ca. 2 mm. Female flowers are in solitary; pedicels 0.5-1.5 mm; 6-lobed calyx campanulate, ca. 4-6 mm in diameter, ca. 1.5-3 mm high; sepals ovate-oblong, enlarged to ca. 12 mm in diam. in fruit; ovary globose; style present, very stout; stigmas with 3 short forked arms, ca. 0.5 mm. Fruiting pedicel 2-6 mm; capsules globose, 5-6 × 8-10 mm, exocarp fleshy, tardily dehiscent, red and ripening brown, apex smooth or with a shallow ring. Seeds 3.6-4 × 2.2-2.8 × 2.2-2.8 mm, yellow to red.

Figure 1: a) *Brenia retusa* Habit b) flowers c) fruits

There are only few reported literature available regarding phytochemical studies on the different parts of *Brenia retusa*. This is an approach to perform the preliminary phytochemical comparative studies among the different parts of *Brenia retusa*. Furthermore qualitative analyses of phenolics were executed using different chromatographic techniques.

Materials and Methods

Collection and authentication of plant material

Breynia retusa (Dennst.) Alston. Collected in August 2017 from Vadodara, Gujarat, India, was identified and authenticated at Department of Botany, The M. S. University of Baroda, Gujarat, India. The voucher specimen of this plant was deposited at the Herbarium, BARO, Department of Botany, The M. S. University of Baroda, Gujarat, India. The plant material was washed, shade dried for a day and then dried completely in an oven at 38 ˚C. The plants were coarsely powdered using a rotary grinder and stored in airtight plastic containers, and then used for phytochemical analysis.

Materials

HPLC grade methanol was purchased from Merck (India) and acetic acid, petroleum ether, sulphuric acid, hydrochloric acid, glacial acetic acid, liquor ammonia, ethyl acetate, n-hexane, acetone, chloroform, perchloric acid were procured from (Spectrochem, India). Ferulic acid (99%), caffeic acid (97%), syringic acid (98%), gallic acid (97%), protocatechuic acid(98%), gentisic acid (98%), veratric acid (99%), p-coumaric acid (98%), o-coumaric acid (99%), m-coumaric acid (98%), sinapic acid (99%), p-hydroxybenzoic acid (98%), vanillic acid (97%), Chlorogenic acid (95%) were purchased from Sigma–Aldrich (India). HPLC water was obtained from Merck (India). Potassium hydroxide, sodium hydroxide, mercuric chloride, potassium iodide, iodine, bismuth carbonate, sodium citrate, sodium carbonate, sodium acetate, α-napthol, copper sulphate, copper acetate, sodium potassium tartarate, lead acetate, ferric chloride were purchased from Sigma Aldrich India. All chemicals and solvents were purchase were of analytical grade.

Experimental Details

Preliminary phytochemical analysis

All the parts (i.e. leaves, stem, root, fruit) were shade dried and coarsely powdered followed by successively extraction with different solvents by using soxhlet apparatus and analysed using simple

chemical tests for preliminary screening of various groups of phytoconstituents such as alkaloids, flavonoids, phenolic acids, sterols, cardiac glycosides, tannins, and so on, as per WHO guidelines [6], [7], [8].

Extraction of phenolics

20g of fine grounded powered of different parts of plant material was separately treated using 2 M HCl and refluxed for 1hr. The whole mixture was cooled, filtered and extracted with ethyl extract. The ethyl acetate layer was concentrated and used for paper chromatography [7], [9] **Paper chromatography**

Paper chromatograms were developed on 25cm X 15cm Whatmann filter paper 1 using 10% acetic acid solution as mobile phase [7], [9].

HPLC sample preparation for Phenolic acids:

The three different bands were separated in the paper chromatographic experiment in which the white florescent band near Rf 0.9 -1.0 containing high amount of phenolic acids. These bands were marked using pencils and each separated band of different plant parts were stripped off and individually extracted with HPLC grade methanol (5 ml). All the samples were sonicated and filtered through 0.22 µm nylon filter. The stock solutions of individual standards (1000 mg) used to prepare the working standard mixtures at the desired concentration. After experimental procedures, the stock solutions and working standards were wrapped in aluminium foil and stored at 4 ^{o}C to prevent photodegradation. For chromatographic analysis, samples and solvents were filtered using 0.22 µm membrane filters [7], [9].

Chromatographic conditions:

The HPLC was conducted using Shimadzu, Kyoto, Japan LC-20AT instrument, equipped with a diode-array detector (DAD) Photo diode array –SPD-M20A Shimadzu, Kyoto, Japan, a binary pump. The reversed-phase C18 analytical column (4.6× 250 mm, 1.8 µm particle size; Hypersil Octadecyl Silicate (ODS)) were used for the chromatographic separation of samples and standards. The phenolic extracts sample of all different parts and standards were separately injected in the system and the injection volume of standards and samples was 20 µL. The mobile phase, consisting of Acetonitrile as solvent A and 1% acetic acid as solvent B, was pumped at 1.0 mL/min into the HPLC system with the following gradient elution program: The applied gradient was 0–10 mins: 100–90% A, 10–20 mins: 90–85% A and hold at 30% A for 5 mins and the flow rate was 0.5 mL min^{-1}. The analyses were performed at 25 ^{o}C, injection volume was 10 µL and the lambda max was set at 280 nm. For identification purposes, the retention, the UV chromatogram parameters of each extract were compared with the standard controls and the peak purity with the UV–visible spectral reference data.

Results and Discussions

The preliminary screening for different phytochemical groups shows presence of various phytochemicals as shown in table 1. The observation shows the presence of almost all phytochemical groups in all parts (i.e. leaf, root, stem and root) of plant except terpenoids. Terpenoidal group test were negative in case of leaf, root and stem extracts but its presence was confirmed in *Brenia retusa* fruit.

Table 1: Results of phytochemical tests performed on different parts of *Brenia retusa*

Phytochemical groups	LEAF	ROOT	STEM	FRUIT
Saponins	+	+	+	+
Phenolics	+	+	+	+
Flavonoids	+	+	+	+
Terpeniods	-	-	-	+
Alkaloids	+	+	+	+
Reducing Sugar	+	+	+	+
Proteins	+	+	+	+
Carbohydrates	+	+	+	+

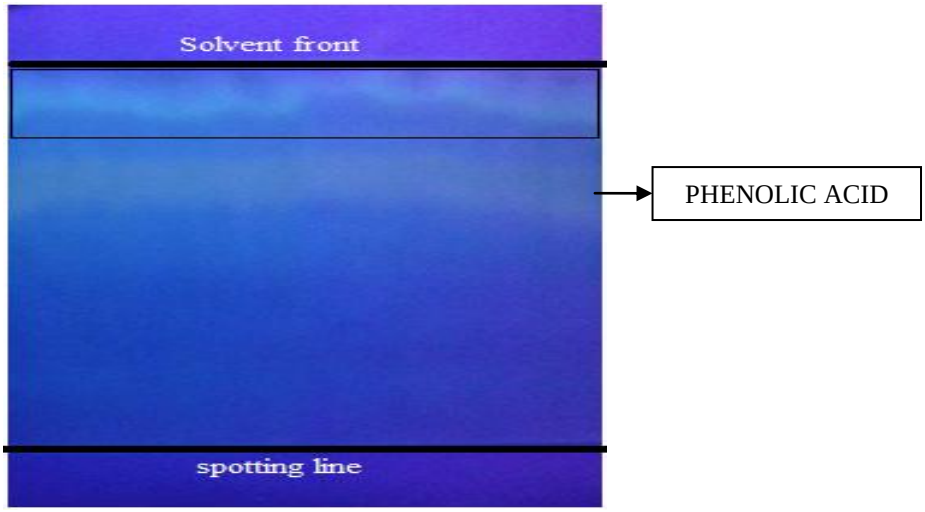

Figure 2: 1-D paper chromatography for different parts of *Brenia retusa*

Phenolic compounds are highly polar in nature. These compounds were confirmed by bluish-white fluorescence with approximately Rf between 0.9 to 1 using 1-D paper chromatography. These bands were of phenolic acid group as in pure form as shown in figure 2. These phenolic groups were directly used for further qualitative analysis of phenolics present.

The sample extracted from the above experiment i.e. 1-D paper chromatography were used in HPLC qualitative analysis of different phenolic acids present in the different parts of *Brenia retusa*.

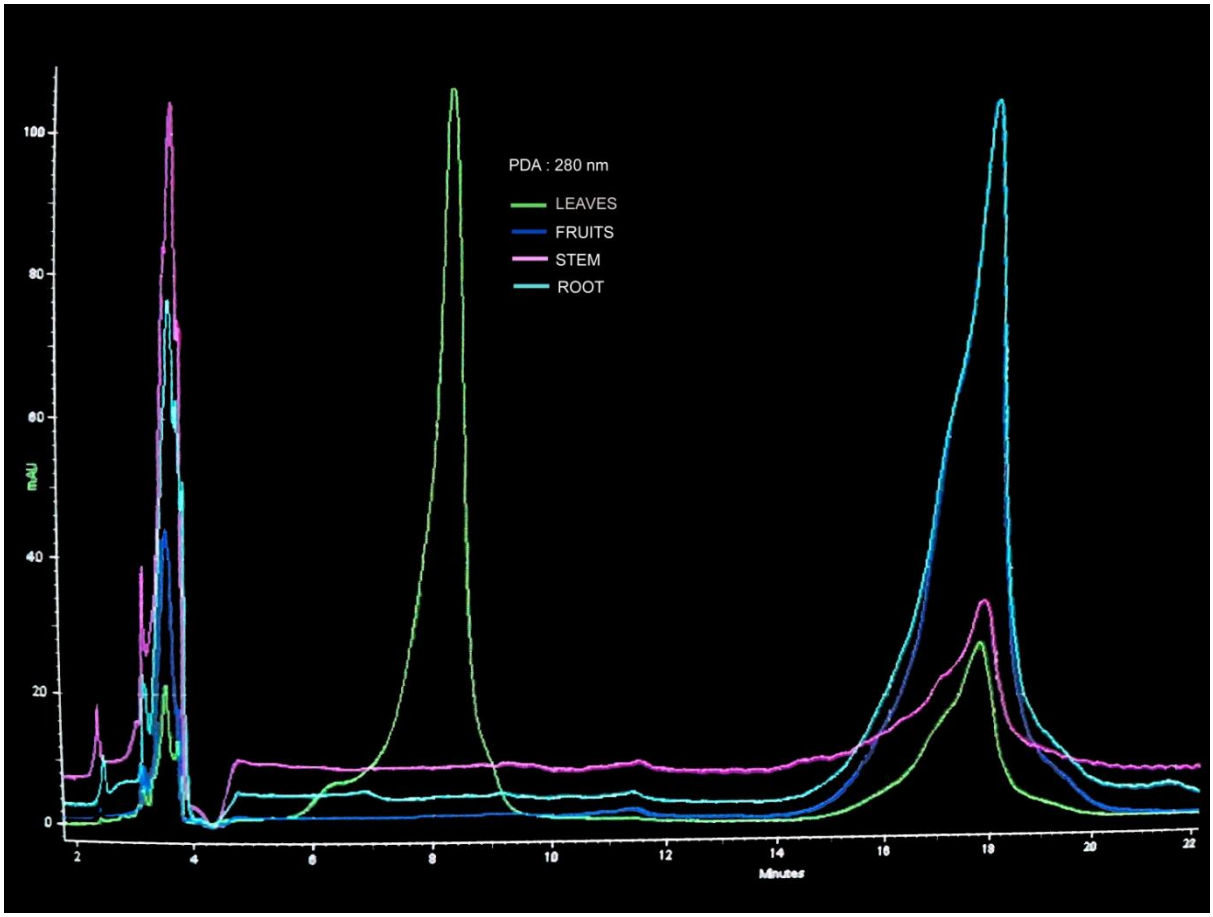

Figure 3: HPLC chromatogram of all 4 parts of *Brenia retusa*

The standard phenolic acids peaks retention time and λmax were compared with the chromatogram of

extracts of different plant parts. The HPLC data revealed the presence of gallic acid only in leaf extract at retention time 8.4 min, λmax = 270 nm. At retention time 17.4 minute, the peak having λmax = 260 nm, 291 nm in all parts of plants (leaves, stem, root, and fruit) showed the presence of Vanillic acid.

Conclusion

A preliminary study shows the richness of phytochemical group in all parts of *Brenia retusa*. Phytochemical groups such as saponins, Phenolics, alkaloids, proteins, carbohydrates, reducing sugar are present in all parts of plants whereas terpenoids are only confined to their fruit extracts.

The chromatographic separation of phenol acid from different parts shows the presence of many phenolics present in different parts of *Brenia retusa.*

HPLC data conclude that *Breynia retusa* leaves contain gallic acid, and vanillic acid as a phenolics, whereas in other parts of plants (stem, root, and fruit) only vanillic acid as a phenolics was identified.

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