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Short Communication

QUALITATIVE AND QUANTITAIVE ESTIMATION OF PHYTOCHEMICALS IN *SKIMMIA ANQUETILIA*: A COMPARATIVE STUDY OF DIFFERENT PARTS

Saduf Nissar*[1], Neelofar Majid[1], Aabid M. Rather[2], Weekar Younus Raja[3], Irshad A. Nawchoo[1], Zulfikar Ali Bhat[3], Kausar Rashid[1]

[1]*Plant Reproductive Biology, Genetic Diversity and Phytochemistry Research Laboratory, Department of Botany, University of Kashmir, Srinagar, Jammu &Kashmir, India*

[2]*Department of School Education, Jammu &Kashmir, India*

[3]*Department of Pharmaceutical Sciences, University of Kashmir, Srinagar, Jammu &Kashmir, India*

**Corresponding author: sadufnissarnaik@gmail.com*

ABSTRACT

Skimmia anquetilia is an evergreen aromatic shrub having potent medicinal importance. Although a few studies have been carried out on phytochemistry of *Skimmia anquetilia* but not much attention has been paid to the variation of phytochemical content in different parts of the plant. Therefore, the present work has been designed to evaluate the variation of phytochemical constituents in different parts (leaves, stem, root and fruits), so that full pharmacological potential of the plant could be exploited. The Soxhlet extraction of different parts of *S. anquetilia* was done and the extracts (hydro-alcholic) were analyzed for qualitative and quantitative estimation of various phytochemicals. The qualitative screening revealed the presence of amazing phytochemicals *viz.,* phenolics, flavonoids, tannins, alkaloids, terpenoids and saponins. The quantitative estimation of phytochemicals depicted a significant variation among different parts and leaves are considered to possess highest quantity of phytochemicals. These phytochemicals could serve as major source for pharmaceutical products, so the plant species could hold an immense potential to serve as therapy for various chronic diseases.

Keywords: Hydro-alcholic, Phytochemicals, *S. anquetilia*.

1. INTRODUCTION

Plants have been a source of natural products as remedies against diseases since human civilizations have been exploring and using plants and their products. About 80% of human population of the world still relies on traditional plant based medicine [1]. In the past two decades, nearly two thirds of approved new drugs were obtained from natural plant products [2] and this plant based therapeutics is gaining popularity both in developing and developed countries as these are natural in origin, have no adverse side effects and are easily available at affordable prices [3]. The medicinal value of plants emanate from secondary metabolites and are produced in huge quantities only when they are needed by the plant, at a particular stage of lifecycle, in certain seasons or in those tissues that require high protection [4]. A wide range of medicinal plant parts such as root, stem, flower, fruit, twigs, exudates and modified plant organs are used as raw drugs and they possess varied medicinal properties. Although various studies have been carried out on phytochemical constituents in many medicinal plants, not much attention has been paid to the variation of phytochemicals in different parts of these plants. So, the present study seeks to provide a comprehensive data on the comparative phytochemical analysis of one of the medicinal charms of Western Himalaya, *S. anquetilia*.

Skimmia anquetilia is an evergreen aromatic shrub, locally known as "Naer" and is endemic to Western Himalaya [5]. It is distributed in the mountain ranges of Kashmir Himalaya confined to shady regions mainly in association with conifers. It is a medicinal plant that has wide importance in conventional system of medicine. In Uttarakhand (India) paste of its leaves with turmeric is used to treat swellings and rheumatism. Its bark powder is used to heal wounds [6]. The roots of this plant are used as an antidote to snake bite and scorpion bite while the dried leaves are used as pesticides and insecticides, in the treatment of fever, cold and headache [7]. The phytochemical profiling of the plant revealed that its

leaves contain linalool, geraniol, pinene, scopoletin, skimmianine, umbelliferone [8]. In view of remedial potential of this plant, the present research work aims at screening different parts of *S. anquetilia* for the presence of potent phytochemicals, so that complete phytochemical potential of the plant could be explored.

2. MATERIAL AND METHODS

2.1. Collection of plant material

Healthy and disease free plants of *S. anquetilia* were collected from Gulmarg area of Jammu and Kashmir. The collected specimens were identified and deposited in Kashmir University Herbarium (KASH) under voucher number 2037-KASH. The plant collections were made quite judiciously throughout the course of the present study. The plant materials were fragmented into different parts (leaves, stem, root, fruit) and dried under shade at room temperature for 15-20 days. After shade drying, the plant materials were powdered and stored under proper conditions for future use.

2.2. Preparation of plant extract

The powdered samples (leaves, stem, root and fruits) of *S. anquetilia and D. albus* were extracted separately in Soxhlet apparatus using hydro-alcohol (70% ethanol) solvent for 2-3 days. The extracts were concentrated under reduced pressure with rotory vacuum evaporator to get viscous masses. Finally the extracts were dried, weighed, labeled and stored at 4°C in storage vials for experimental uses.

2.3. Phytochemical screening

The hydro-alcholic extracts of *S. anquetilia* were screened for different phytochemicals. These include some primary metabolites- carbohydrate, proteins, amino acids, glycosides and cardiac glycosides; and major secondary metabolites- alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids. The phytochemical screening was undertaken following standard methods [9-23].

2.4. Quantitative estimation

2.4.1. Alkaloid determination

Powdered samples (2.5 g) were extracted using 100 ml of 20% acetic acid in ethanol and covered for almost 4 hours. Filtrate was concentrated to 25 ml. Concentrated ammonium hydroxide was added stepwise to achieve precipitation. The whole solution was kept as such to allow settling of precipitate. Collected precipitates were washed with dilute ammonium hydroxide and finally filtered. Filtrates were disposed off and pellet obtained were dried and weighed [24, 25].

2.4.2. Saponin determination

Ten (10) g of samples were mixed with 100 ml of 20% aqueous ethanol. The mixture was kept at 55°C on water bath shaker for 4 hours. Filtrates were extracted in the same way again. The combined extracts were concentrated over water bath at 90°C to 40 ml. Concentrates were transferred to a separating funnel followed by the addition of 10 ml diethyl ether. After vigorous shaking, aqueous layer was recovered and the ether layer discarded. The process was repeated and n-butanol was added to the aqueous layer. The entire mixture was washed 10 ml 5% of aqueous NaCl in a separating funnel twice. Upper part was retained and heated until evaporation in water bath. Later it was dried in oven to a constant weight [24, 26].

2.4.3. Terpenoid determination

Ten (10) grams of plant powder was soaked in alcohol for 24 hours and then filtered. The filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids [27].

2.4.4. Phenolics determination

Total phenolic content was determined by spectrophotometric method. For the analysis, 1mg/1ml of the plant extract solutions were prepared. 0.5ml and 1 ml of the solutions were transferred to separate test tubes followed by addition of 1ml of Folin-Ciocalteu reagent (2N) and 4ml of 20% of Na_2CO_3 and ultimately the volume was made up to 6ml with double distilled water. The samples were vigorously shaken and finally allowed to incubate at room temperature for 2 hours after which the absorbance was taken at 765 nm. The samples were prepared in triplicates. Quantification was done using standard calibration curve of gallic acid. The results were expressed as of GAE (gallic acid equivalents) [28].

2.4.5. Flavonoid determination

Total flavonoid content in the plant extracts were quantified using spectrophotometric method. The sample solutions of the extracts were prepared in the concentration of 1 mg/ml. To the test solutions of various concentrations 1 ml of 2% $AlCl_3$ solution (dissolved in methanol) was added. The samples were incubated at room temperature for one hour and finally the absorbance was recorded at 415 nm. For each

analysis, samples were prepared in triplicate and the mean value of absorbance was obtained. The same methodology was followed for the standard solution of rutin and the calibration curve was construed. The flavonoid content in the extracts was expressed in terms of rutin equivalent (mg of RU/g of extract) [29].

2.4.6. Tannin determination

The Folin-Ciocalteu protocol was followed for the determination of total tannin content. The plant extracts were prepared in the 1mg/ml concentrations. A dilution of the sample extracts were taken in test tubes followed by the addition of 7.5 ml of distilled water, 0.5 ml of Folin-Ciocalteu reagent and 1 ml of 35 % Na_2CO_3 solution. Final volume (10 ml) was made with distilled water. The reaction mixtures were vigorously shaken and incubated for 30 minutes at room temperature. The same protocol was followed for standard solutions of gallic acid. Absorbance was measured against the blank at 725 nm with an UV/Visible spectrophotometer. The tannin content was expressed in terms of mg of GAE /g of extract [30].

3. RESULTS AND DISCUSSION

3.1. Phytochemical screening

Plants are essential natural resources which constitute one of the potential sources of new products and bioactive compounds which could serve as newer leads and clues for modern drug design [31, 32]. The results of qualitative phytochemical screening are summarized in Table 1. The study revealed the presence of a number of phytochemicals in different parts of *S. anquetilia* (Table 1). However, both anthraquinone and cardiac glycoside did not show their presence in any of the parts of *S. anquetilia*.

Table 1: Qualitative analysis of phytochemicals in hydro-alcholic extract of different parts of *Skimmia anquetilia*

Phytochemicals	Tests	Presence/absence in different parts of the plant			
		Root	Stem	Leaves	Fruit
Carbohydrates	Molisch's	+	+	+	+
	Benedict's	+	+	+	+
Anthraquinone glycosides	Borntrager's	-	-	-	-
Cardiac glycosides	Keller killiani	-	-	-	-
	Legal's	-	-	-	-
Proteins and amino acids	Xanthoproteic acid	+	+	+	+
	Ninhydrin	+	+	+	+
Alkaloids	Dragendroff's	+	+	+	+
	Wagner's	+	+	+	+
	Hager's	+	+	+	+
Phenolics	Ferric chloride	+	+	+	+
Tannins	Ferric chloride	+	+	+	+
	Lead acetate	+	+	+	+
Flavonoids	Shinoda	+	+	+	+
	Alkaline reagent	+	+	+	+
Terpenoids	Salkowski	+	+	+	+
Saponins	Foam	+	+	+	+

+ indicates presence, - indicates absence

3.2. Quantitative estimation

All plants produce chemical compounds as part of their normal metabolic activities. Plants synthesize a wide variety of chemical compounds, which can be sorted by their chemical class, bio synthetic origin and functional groups into primary and secondary metabolites [33]. These phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds [34]. The results revealed that the quantity of various phytochemicals showed a significant variation ($p \leq 0.05$) among different parts of the plants. The detailed results are summarized in Table 2, Fig.1.

3.3. Total alkaloid content

The alkaloids are nitrogen-containing secondary metabolites found in approximately 20% of the vascular plant

species [35]. In *S. anquetilia*, leaves (101.33± 0.153mg) showed more alkaloid content followed by stem (96.43±0.666mg), fruit (90.20±0.436mg) and root (74.47±0.404mg). The higher alkaloid content in leaves could be attributed to the fact that alkaloids have an important role in anti-herbivory phenomenon. As leaves are the first prey to animals so the highest content was also reported in leaves as a protective measure. In plants the alkaloids are often used as protection against animals, as many of them are toxic by affecting neurotransmission [36].

Table 2: Quantification of major phytochemicals in different parts of *Skimmia anquetilia*

Phytochemical content	Parts				**F	***P
	Leaves	Stem	Root	Fruit		
Alkaloids (mg/g)	101.33±0.153[a*]	96.43±0.666[b]	74.47±0.404[c]	90.20±0.436[d]	1998.42	0.000
Saponins (mg/g)	40.26±0.814[a]	11.40±0.435[b]	6.43±0.416[c]	18.46±0.253[d]	2451.95	0.000
Terpenoids (mg/g)	65.76±0.153[a]	37.50±0.458[b]	26.73±0.115[c]	33.53±0.473[d]	7530.827	0.000
Phenolics (mg of *[1]GAE/g)	397.00±1.026[a]	373.58±2.163[b]	302.19±0.983[c]	344.96±1.187[d]	2463.740	0.000
Tannins (mg of GAE/g)	43.94±1.720[a]	33.16±1.233[b]	10.78±0.980[c]	30.06±0.748b	381.778	0.000
Flavonoids (mg of *[2]RU/g)	35.97±1.405[a]	19.78±0.972[b]	7.783±1.172[c]	23.20±1.501[d]	246.239	0.000

***** *Means labeled with the different small letters indicate that they significantly differ from each other among different parts, **F-test (ANOVA), ***Level of significance (p≤0.05), *[1]Gallic acid equivalent, *[2]Rutin equivalent*

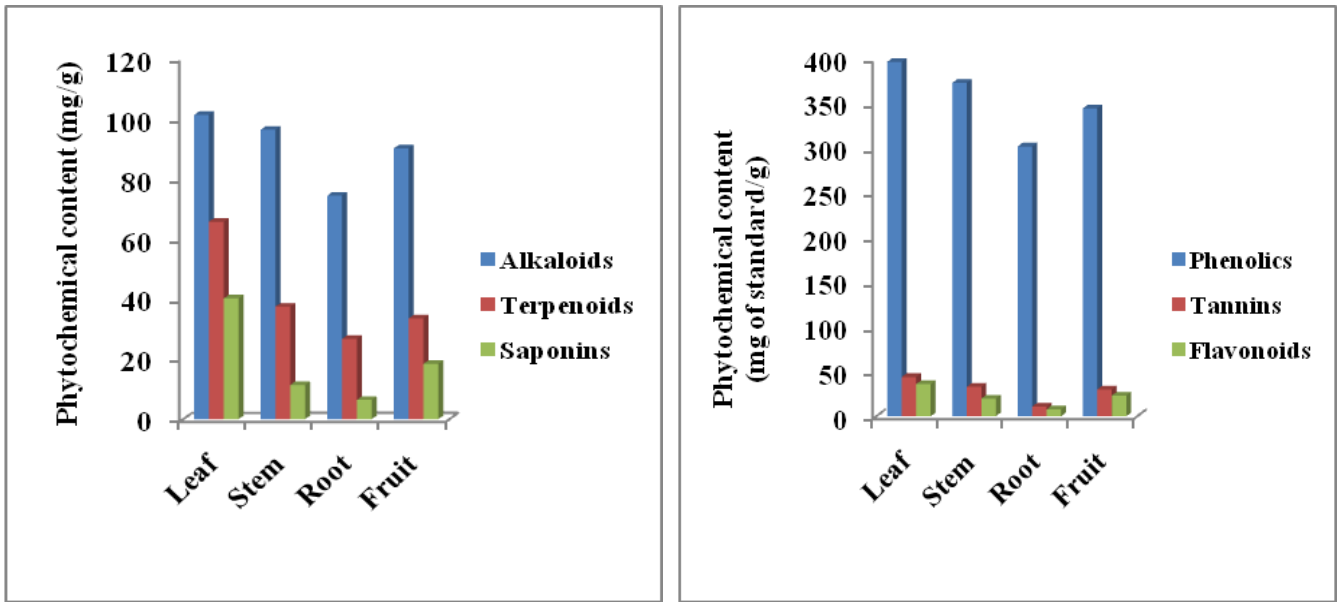

Fig. 1: Phytochemical variation in different parts of *Skimmia anquetilia*

3.4. Total saponin content

Saponins are high-molecular-weight secondary metabolites consisting of triterpenoidal or steroidal aglycone, having a wide distribution in the plant kingdom [37]. Triterpenoid saponins are found principally in dicotyledons while steroidal saponins occur in monocots [38]. The main function of saponins is to provide protection against many pathogens and herbivores. The possible mechanism of action of saponins is membrane perturbation [39]. Some studies advocated that in plants, variations in saponin distribution, composition and amounts may influenced by the surrounding environment, growth stage and varying needs for plant protection [40, 41]. The present investigation revealed that the total saponin content does not show any typical trend. In *S. anquetilia*, it ranges from 6.43±0.416mg (root) to 40.26±0.814 (leaves).

3.5. Total terpenoid content

Terpenes constitute the largest and most diverse group of natural products. Some plant families are known for their capability to synthesize and emit antimicrobial terpenes as a defensive measure against microbial challenges [42, 43] and to attract specific insects for

pollination [44]. Plants that occupy resource-limited habitats are known to secrete relatively constant amount of terpenes into their rhizosphere for competing other plant species [45]. The higher terpenoid content was observed in the leaves and lower in the root extracts and ranges from 26.73±0.115mg (root) to 65.76± 0.153mg (leaves).

3.6. Total Phenolic content

Phenolics are one of the most ubiquitous groups of secondary metabolites found throughout the plant kingdom [46]. A number of simple and complex phenolics act as phytoalexins, phytoanticipins and nematicides against soil-borne pathogens and phytophagous insects [47, 48]. These also provide structural integrity and scaffolding support to plants. Plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. While taking the total phenolic content of *S. anquetilia* into consideration, leaves were found to have highest content (397.00±1.026 mg) as compared to the other parts.

3.7. Total tannin content

Tannins are medicinally important because of their astringent properties. They are also known as proanthocyanadins possessing significant properties like antioxidant, anti-apoptosis, anti-aging, anti-carcinogenic, anti-inflammatory and antiartherosclerosis [49]. The anti-carcinogenic and anti-mutagenic potentials of tannins may be related to their anti-oxidative property, which is important in protecting cellular oxidative damage, including lipid peroxidaton [50]. It was observed that total tannin content showed a huge variation among different plant parts. The highest content was observed in the leaf (43.94±1.720mg) and least content in root (10.78±0.980mg) extract of *S. anquetilia*.

3.8. Total flavonoid content

Flavonoids represent the most common and widely distributed class of plant phenolics [35]. The interest in plants rich in flavonoids has tremendously intensified due to their potentially beneficial effects in humans *viz.*, anti-allergic, anti-viral, anti-inflammatory, anti-platelet, anti-tumor, and anti-oxidant activities [51]. The total flavonoid content showed a huge variation between the plants studied. The highest content was shown by the leaf (35.97±1.405) extract of *S. anquetilia*.

4. CONCLUSION

The findings of this study indicate the presence of various phytochemicals in the plant extracts, which may be responsible for the pharmacological activity of this plant. From the results obtained by the present study it becomes clearly evident that all the parts contain natural products but in varied quantities wherein leaves can be considered as potent phytochemical factories. Hence leaves could be used as a good source for the isolation of compounds.

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