



Physicochemical and Preliminary Phytochemical standard of leaves of *Skimmia anquetilia*

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Abstract

Skimmia anquetilia (Rutaceae) is an evergreen shrub. They originate from the Himalayas in Kashmir in India and spread right across south-east Asia, China and Japan. The current communication attempts to evaluate the physicochemical and preliminary phytochemical studies on the leaves of *Skimmia anquetilia*. This study would be of enormous value in the pharmacognostical identification and standardization of the drug in crude form and would help to distinguish the drug from its other species. The percentage of total ash value, acid insoluble ash value, water soluble ash value and sulphated ash value were obtained as 16.86, 4.38, 10.66 and 8.11% respectively. Fluorescence characters of different extracts with various reagents were noted under UV (shorter wavelength-254nm and longer wavelength- 365nm) and under normal ordinary light. Loss of weight on drying and foreign organic matters was found as 4.28 and 0.21 % w/w respectively. The percentage yield of successive leaf extracts using different solvents as petroleum ether, chloroform, ethyl acetate, methanol and distilled water were found as 6.06%, 4.40%, 3.18%, 18.54%, and 17.16% w/w respectively. The preliminary phytochemical screening was carried out for the presence of alkaloids, flavonoids, carbohydrate glycosides, tannins, terpenoids, phenol, steroids and saponins for different extracts of leaves of *Skimmia anquetilia*. The present study provides referential information for proper identification and standardization of this plant material.

Keywords: *Skimmia anquetilia*, physicochemical, florescent behaviors, pharmacognostical analysis, phytochemicals

Introduction

Skimmia anquetilia is an aromatic gregarious shrub of the Rutaceae family commonly known as Kedarpatti in Hindi (Negi *et al.*, 2011) ^[1]. They originate from Western part of Himalayas and Kashmir in India and spread right across south-east Asia, China and Japan (Watanabe, 2002). The leaves are dark green and ovate in appearance. Traditionally, the leaf infusion of *Skimmia anquetilia* is taken for treatment of headache, freshness and general fever (Bhattarai, 1992) ^[2]. The leaves of *Skimmia anquetilia* are aromatic and known to contain linalool, geraniol, pinene, scopoletin, skimmianine, umbelliferone (Kunwar, 2010) ^[4].

Plant material and method

The leaves of *Skimmia anquetilia* were collected from Sonamarg area of Kashmir (J&K, India). *Skimmia anquetilia* was properly identified (Voucher specimen-KUCS07) by Dr. A. R. Naqshi, Taxonomist, Department of Pharmaceutical Sciences, University of Kashmir, Srinagar. The collected leaves were shade dried and coarsely powdered. The coarse powder was subjected to continuous extraction in a soxhlet apparatus separately using petroleum ether, chloroform, ethyl acetate, methanol and distilled water as solvents.

Preparation of the successive leaf extracts

The leaves of *Skimmia anquetilia* (S. A.) were dried under shade, separated and crushed made to form dry powder. Dried and crushed 500 g of leaves of *Skimmia anquetilia* first defatted using petroleum ether (60-80°C) in soxhlet apparatus to remove fatty substances. The defatted marc was collected

and dried after the completion of defatted process. Defatted marc was subjected to successively continuous hot extracted with (increasing polarity manner) chloroform, ethyl acetate, methanol and distilled water to prepared petroleum ether (PE), chloroform extract (CE), ethyl acetate extract (EAE) methanol extract (ME) and distilled water extract(AE). After complete extraction, the solvents were evaporated from each extract individually and concentrated to dry residue (Brindha, 1981) ^[5]. All the extractive values of the leaves of *Skimmia anquetilia* with different solvents were obtained are calculated and showed in the table 1.

Table 1: Successively extractive values of different leaves extracts of *Skimmia anquetilia*.

S No.	Nature of extracts	%Yield (w/w)	Color of the extractive
1.	Petroleum ether(6080°C)	6.06	Light brown
2	Chloroform	4.40	Brown
3	Ethyl acetate	3.18	Light brown
4	Methanol	18.54	Violet
5	Aqueous	17.16	Dark brown

Evaluations of physico- chemical parameters

Physicochemical constants were evaluated for the purpose of standardization such as the percentage of total ash, water soluble ash; acid insoluble ash, sulphated ash, loss on drying and total extract values were calculated according to standard procedures. (Pharmacopoeia, 1996; Kokate 1994) ^[6, 7]. The values of physicochemical constants were calculated and showed in the table 2. The physicochemical constant

determines as above references and procedures as followings subheadings:

Table 2: Physicochemical parameters of leaves extracts of *Skimmia anquetilia*.

S. No.	Parameters	Content
1	Foreign organic matter	0.21%
2	Methanol soluble extractive	34.83%
3	Water soluble extractive	32.67%
4	Total ash	16.86%
5	Acid-insoluble ash	04.38%
6	Water soluble ash	10.66%
7	Sulphated ash	08.11%
8	Loss on drying	04.28 %

Determination of ash values

The determination of ash values is meant for detecting low-grade products, exhausted drugs and sandy or earthy matter. It can also be utilized as a mean of detecting the chemical constituents by making use of water-soluble ash and acid insoluble ash.

Total ash

Accurately about 3 gms of air dried leaves powdered drug was weighed in a tarred silica crucible and incinerated at a temperature in range 440-460 °C until free from carbon, cooled and weighed and then the percentage of total ash with reference to the air dried powdered drug was calculated. The percentage of total ash with reference to the air-dried drug was calculated.

Acid insoluble ash

The total ash obtained was boiled for 5 minutes with 25ml of dilute HCl and filtered it. The residue was collected on ash less filter paper and washed with hot water; filter paper with residue was ignited and weighed. The percentage of acid insoluble ash was calculated with reference to the air dried drug was calculated.

Water soluble ash

The total ash obtained was boiled for 5 minutes with 25 ml of distilled water. The insoluble matter was collected on an ash less filter paper, washed with hot water and filter paper with residue was ignited to constant weight at a low temperature. The weight of insoluble matter was subtracted from the weight of the ash. The difference in weights represents the water soluble ash. The percentage of water soluble ash with reference to the air dried drug was calculated.

Fluorescent behavior of powder

In fluorescence analysis (Khandalwal, 1999) ^[9] the powdered sample were treated with various chemical reagents like aqueous 10% Sodium hydroxide, alcoholic 1N sodium hydroxide, 1N hydrochloric acid, 50% sulphuric acid and concentrated nitric acid, picric acid, acetic acid, ferric chloride, conc.HNO₃ + NH₃ etc. and their extracts were subjected to fluorescence analysis in day light and UV light (254 nm and 365 nm).The observed values of fluorescence analysis are showed in table 3.

Table 3: Fluorescence characteristics of the powdered sample leave extracts of *Skimmia anquetilia*.

Sl. No	Treatment	Visible/Day light	Short UV light (254 nm)	Long UV light (365 nm)
1	Powder as such	Light green	Fluorescent green	Brown
2	Powder + 50% H ₂ SO ₄ + water	Darkest brown	Brown with green fluorescent	Brown with milky white fluorescent
3	Powder + Con. Hcl	Fluorescent green	Green	Reddish brown
4	Powder + Con. Hcl+ water	Light green	Green	Light brown
5	Powder + Con. HNO ₃	Reddish brown	Green	Light brown
6	Powder + Con. HNO ₃ + water	Light green	Green	Brown
7	Powder + Acetic acid	Dark green	Green	Light brown
8	Powder + Methanol	Dark green	Light green	Brown
9	Powder + Chloroform	Dark green	Green	Brown
10	Powder + Petroleum ether	Light green	Dark green	Light brown
11	Powder + Distilled water	Light green	Fluorescent green	Brown
12	Powder + 10% NaOH (aqua.)	Yellowish green	Fluorescent green	Light brown
13	Powder + 5% iodine	Dark brown	Green	Black
14	Powder + 10% Picric acid	Yellowish green	Fluorescent green	Violet
15	Powder +5% Ferric chloride	Muddy green	Intense green	Black
16	Drug Powder + NH ₃ solution	Green	Fluorescent green	Light brown
17	Powder + Ethyl acetate	Green	Light green	Light brown
18	Powder + 1N NaOH(alcoholic)	Light Yellow	Green	Brown
19	Powder + Conc. H ₂ SO ₄	Light brown	Dark green	Brown with white fluorescent

Determination of moisture content (Loss on drying)

Place about 10 g of drug (without preliminary drying) after accurately weighing in a tarred evaporating dish and kept in oven at 105⁰ C for 5-6 hours for constant weigh. The percentage loss on drying with reference to the air dried drug was calculated.

Determination of foreign organic matter

Accurately weighed 100 g of the drug sample and spread it out

in a thin layer on white board sheet. The foreign matter should be detected by inspection with the unaided eye or by the use of a lens (10X). Separate and weigh it and the percentage present was calculated.

Determination of extractive values

a) Alcohol Soluble Extractive

5 gm of coarsely powdered air dried drug was macerated with 100 ml of alcohol in a closed flask for 24 hour, shaking

frequently for six hours and allowed to stand for 24 hours. It was then filtered rapidly taking precaution against loss of alcohol. 25 ml of the filtrate aliquot was evaporated to dryness in tarred flat bottomed shallow dish, dried at 105^oc and weighed. The percentage of alcohol soluble extractive was calculated with reference to the air dried drug.

b) Water Soluble Extractive

5 gm of coarsely powdered air dried drug was macerated with 100 ml of chloroform water in a closed flask for 24 hours, shaking frequently for six hours and allowed to stand for eighteen hours. It was then filtered rapidly taking precautions against loss of chloroform water. 25 ml of the filtrate was evaporated to dryness in tarred flat bottomed dish dried at 105^oc and weighed. The percentage of water soluble extractive was calculated with reference to air dried drug.

c) Ether soluble extractive

100 gm of coarsely powdered air dried drug was extracted with solvent ether for six hours. The extract is filtered into a tarred evaporating dish and evaporates off the solvent on a water bath. The residue is dried at 105^oC to constant weight. The percentage of ether extractive was calculated with reference to air dried drug.

Preliminary phytochemical analysis

Successively obtained materials were filtered and concentrated using vacuum distillation. The different leaves extracts were subjected to qualitative tests for the identification of various phytochemical constituents as per the standard procedure (Harbone, 1998) ^[8] showed in the table 4.

Table 4: Preliminary phytochemicals screening of leaves extracts of *Skimmia anquetilia*.

S. No.	Test	P.E.	C.E.	E.A.E.	M.E.	A.E.
Carbohydrates-						
1.	Molish test	+	+	+	++	++
	Fehling's test	+	-	-	+	+
	Benedict test	-	-	-	++	+
	Seliwnoffs test	-	-	-	-	-
Alkaloids						
2	Mayer's test	-	++	+	++	+
	Hager's test	-	-	-	++	+
	Dragendroffs test	+	++	-	++	+
Saponins						
3	Foam test	-	-	+	++	++
Steroids						
4	Salkovaski test	+	-	-	++	+
5	Fats and oils					
	Filter paper test	+	-	+	-	-
Proteins						
6	Millon's test	-	-	-	+	++
	Biuret test	+	-	+	+	++
Amino acid test						
7	Ninhydrine test	-	-	-	+	++
Glycosides						
8	Anthraquinone test	-	-	-	-	+
	Keller kiliani test	+	-	++	++	++
Starch						
9	Iodine test	-	-	-	-	+
Tannins and Phenolic						
10	Ferric chloride test	-	-	-	++	++
	Lead acetate test (10%)	-	+	+	++	++
Resins						
11	Ferric chloride test	-	-	-	+	+
Flavonoids						
12	Shinoda test	-	-	+	++	++
	Lead acetate test	-	-	-	+	+

Results

The result of present study supported for physicochemical and preliminary phytochemical standard of leaves of *Skimmia anquetilia*. The preliminary phytochemical tests were performed which showed that leaves extracts of *Skimmia anquetilia* is credited with different secondary metabolites such as alkaloids, glycosides, tannins, phenolics compounds, flavonoids, steroids, saponins, proteins, amino acids and

carbohydrates. The percentage of total ash value, acid insoluble ash value, water soluble ash value and sulphated ash value in leaves powder of *Skimmia anquetilia* were found as 16.86, 4.38, 10.66 and 8.11% respectively. Foreign organic matter in the leaves powder was 0.21%. Loss of weight on drying was found as 3.58 % w/w, which is not too high, hence could discourage bacterial, fungal, or yeast growth (Kumar *et al*, 2011). The percent yield of successive leaves extract of

petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts were found as 6.06%, 4.40%, 3.18%, 18.54%, and 17.16% w/w respectively. Extractive value was highest in methanol and aqueous extracts indicating the possibility of considerable amount of polar compounds in leaves of *Skimmia anquetilia*

Conclusion

Physicochemical constants such as ash values is useful in determining authenticity and purity of drug and also for quantitative standards as reference (Kumar *et al.*, 2011). In this study total ash value is considerably low, which may be due to low content of carbonates, phosphates, silicates and silica. Study of foreign organic matters is useful in distinguishing between similar drugs or in the detection of adulteration. It also helps to remove the more resistant parts of plant organs which can be used for microscopic examination. Fluorescence behavior of drugs sample is an important character exhibited by various chemical constituents present in plant material. Some plant constituents have unique tendency to show fluorescence in the visible range in day light. The ultra violet light produces fluorescence in many natural products (e.g. alkaloids like berberine), which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents hence some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation (Kumar *et al.*, 2011). Preliminary phytochemical screening of different leaves extracts known to possess various secondary metabolites. These secondary metabolites shown various pharmacological effects and may be responsible for various pharmacological effects of leaves of *Skimmia anquetilia*.

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