Middle-East Journal of Medicinal Plants Research 1(3): 51-58, 2012
ISSN 2309-8929

DOI: 10.5829/idosi.mejmpr.2011.1.3.1112

Physicochemical Characterization of Essential and Fixed Oils of *Skimmia laureola* and *Zanthoxylum armatum*

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Abstract: This paper reports the isolation and characterization of essential oil and fatty acids of *Skimmia laureola* and *Zanthoxylumarmantum*. The fatty acid was extracted from the fruit of *Zanthoxylumarmatum*with *n*-hexane and petroleum ether using Soxhletapparatus. The essential oil was extracted from the leaves of *S. laureola* and *Z. armatum* through hydro-steam distillation using Modified Clevenger type apparatus. *Zanthoxylum armatum* fruit showed 9.40 and 9.97% yield of fixed oil while *Z. armatum showed* 0.395 and 0.198% yield of essential oil. Some of the physico-chemical properties of the extracted essential oil and fixed oil such as colour, odour, %, refractive index, specific gravity, carbon residue, absolute viscosity, kinematic viscosity, total acid number, iodine number and saponification value are given in table 1. The present research work suggestsexploring the title plants for chemical constitutions and pharmacological action.

Key words: *Skimmia laureola* · *Zanthoxylum armatum* · Essential oil · Physico-chemical

INTRODUCTION

*Skimmia laureola*is evergreen strong-scented shrub, up to 1 m tall bearing grayish green dichotomous branches. Leaves whorled in terminal clusters. It grows at altitude of 1800-3000 meter under shady condition in forest. It is common in the Hazara region, Murree Hills, Kashmir, Upper Swat and Upper Dir [1, 2]. In Nathiagally the plant is growing gregariously around the tract leading to Mukshpuri top. *S. laureola* is considerd an important medicinal and sacred plants, almost all around its nearby areas. Leaves are used traditionally in local ailments. When crushed, the leaves give a musky odor due to the presence of a poisonous compound skimnianine [1]. Traditionally, burning smoke from the dried leaves and branches is demon repellent. Medicinally they are used for relief in coughs [3]. Leaves are commercially harvested and are used in food as flavoring agent, in traditional healing and cultural practices, being made into garlands and considered sacred Crushed leaves with wheat flour are used as anthelmintic for livestock [1]. The smoke from the leaves is also used for clearing the nasal tract. It is also used as cold remedy, antipyretic and analgesic. The leaves are used as insecticides and pesticides [4].

*Zanthoxylum armatum*is a small tree or large spiny shrub with compound leaves, blooming in March April, prefer semi shady or no shade for their growth. Locally it is called Dambara (Pashtu), Dambrary, Tamur (Urdu) and wing leaf prickly ash (English) and traditionally it is used as a tonic, carminative, condiment, stomachic, anthelmintic [5], Insecticidal [6], for tootheache, abortifacient, antifertility agent [7], antiseptic, disinfectants, deodorant [8] antipyretic and anti diarrheal. It improves speaking power and increase saliva secretion [9]. Fruits and seeds of this plant are used in fever, dyspepsia and skin diseases [10]. In the present study essential oils obtained from the leaf of *S. laureola* (SVO) and *Z. armatum* (ZVO) and fixed oil from the fruit of *Z. armatum,* extracted with different solvent i.e *n*-hexane (ZHO) and petroleum ether (ZEO) were evaluated for physicochemical characteristics.

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Study of various physicochemical characteristics explores the practical importance of herbal oils in daily life. Physicochemical properties of oil like colour, odour, density, specific gravity, refractive index, optical rotation, acid value, iodine value, saponification value etc indirectly influence the quality of both essential and fixed oils. The commercial importance of oils mostly depends on these physicochemical properties, which provide baseline data to determine its suitability for consumption [11, 12]. Viscosity is a measure of resistance of a fluid to deform under shear stress. It is commonly perceived as thickness, or resistance to pouring. Viscosity describes a fluid's internal resistance to flow and may be thought of as a measure of fluid friction. It determines the rheological proprieties of these oils [13].

The refractive index is the degree of the deflection of a beam of light that occurs when it passes from one transparent medium to the other. It increases with the length of chains and with the number of carbon atoms present. Therefore, the refractive index determines evidences that the sample might be unsaturated long carbon chain [14].

The iodine value is a useful tool in predicting the drying properties of oils [15]. The high iodine value of oils indicate the high content of unsaturation and suggests that the oils may be used as drying agent for the manufacturing of oil paints, varnishes, cosmetics and also as cocking oil manufacturing index [16]. The iodine value is also an index of assessing the ability of oil to go rancid. It is also used for determining the level of oxidative deterioration of the oil by enzymatic or chemical oxidation [17].

Acid value is an important physicochemical property index of oil which is used to determine the quality, age, edibility and suitability of oil for industrial use such as paint [18]. This value is used to measure the extent of glycerides in the oil, which have been decomposed by lipase and other physical factors such as light and heat [19].

Saponification value is an index of average molecular mass of various fatty acids in oil samples. The lower value of saponification means molecular weight of fatty acids is lower and has lower limit of use in industry [20]. The saponification value suggests the use of oil in production of liquid soap, shampoos and lather shaving creams [21]. In continuation of our research work on Pakistani medicinal plants [22-29] herein we are reporting physicochemical characterization of essential and fixed oils of *Skimmia laureola* and *Zanthoxylum armatum*.

MATERIALS AND METHOD

Fixed Oil Extraction: Respective powder of *Zanthoxylum armatum* fruit were soaked separately in 1-2 liters of *n*-Hexane and petroleum ether for 12-15 days with occasional shaking. The extracts were then filtered and concentrated with the help of rotary evaporator. Each of these processes were repeated thrice and at the end all the three extracts of each drug were combined. The fixed oil was then separated using standard methodology through both solvent [30].

Essential Oil Extraction: A Modified Clevenger type apparatus were used for the extraction of essential oil from the leaves of *Skimmia laureola* and *Zanthxylum armatum* through hydro-steam distillation. The leaves were thoroughly washed, cut into small pieces, placed in distillation flask and subjected to hydro-steam distillation for about 4 hours. The steam and vaporized oil were condensed into liquid by a vertical condenser and collected in measuring tube. Being immiscible and lighter than water, the volatile oil separated out as an upper layer. The oil was then separated from water and collected in small bottles, dried with anhydrous sodium sulphate, sealed, labeled and stored in light resistant vials at 4-6°C for further use [31].

Physicochemical Characteristics of Oil: Physicochemical characteristics provide a base line for suitability of oils [12]. The physicochemical properties of the oil determined were color, odor, % yield, density, optical activity, refractive index, specific gravity, carbon residue, absolute viscosity, viscosity index, kinematic viscosity, total acid number, iodine number and saponification value.

Color Determination: Color of the respective oils was determined by physical observation in day light and under ultraviolet radiation of 254 and 366 nm using ultra violet chamber [11].

Odor Determination: Odor of the respective oils was determined by organoleptic evaluation following Evans [32].

Determination of Percentage Oil Yield: The percentage oil yield was calculated by using following relation (AOAC, 2000).

$$Percentage\,oily\,yield\left(W/W\right)=\frac{Weight\,of\,oil}{Total\,weight\,of\,materials\,used\,for\,oil\,extraction}\times 100$$

Determination of Optical Rotation: 10ml Polari meter tube containing oil was placed in the trough of the instrument between polarizer and analyzer. Care was taken in filling the tube to avoid the air bubble formation which could disturb the rotation of light. Analyzer was slowly turned until both the halves of the field were viewed through the telescope. The direction of rotation was determined, if the analyzer was turned counter clock wise from the zero position to obtain the final reading, the rotation is levo (-) if clock wise and dextro (+) if anti clockwise [33].

Determination of Refractive Index: The refractive index of the oil samples was determined with the help of Abbe refractometer model A 80251 (BS). Two drops of respective oil were placed on the prism with the help of syringe and the prism was firmly closed by tightening the screw head. The apparatus was allowed to stand for 5 min, after that reading was recorded from the display screen [33].

Determination of Specific Gravity: For the determination of specific gravity of oils, a clean 50 ml specific gravity bottle was weighted (W_0). Then the bottle was filled to the brim with water and stopper was inserted. Extra water spilled out. The water on the stopper and bottle were carefully wiped off and reweighed (W_1). Same process was repeated, but using oil samples instead of water and weighted again (W_2). The specific gravity of the all oil samples were calculated using the following formula [34].

$$Specific\,gravity\,of\,test\,sample=\frac{w_2-w_0}{W_{1-W_0}}$$

Where
W_0 = Weight of empty specific gravity bottle
W_1 = Weight of water + specific gravity bottle
W_2 = Weight of test sample + specific gravity bottle.

Carbon Residue: A sample of known amount was taken in a silica crucible, heated strongly till the vapoures and smoke disappeared, in a sheath iron hood. The sample was then cooled down in a desiccators and cooled down. Carbon residue was then calculated by the following formula (AOAC., 2000).

$$Carbon\,residue\left(\%\right)=\frac{w_1}{w_2}\times 100$$

Whereas
W_1= Carbon residue in crucible
W_2= Weight of sample

Determination of Viscosity: Viscosity is the resistance to the flow and it was determined by using viscometer. Viscosity plays an important role in determining the structure of liquids. The viscosity of the respective oil was determined by using viscometer with a selection of spindle number four which was properly fixed to the holder. The container having the oil was carefully placed below the rotor holding the spindle. The spindle was allowed to immerse into the oil inside the container. The meter was turned on and adjusted to a speed of 6 m/s. then the spindle was allowed to rotate in the oil for a period of 30 min until stable reading displayed on the meter's display screen. The viscosity value of the oil was measured in centipoises [34].

Kinematic Viscosity: Kinematic viscosity is the ratio of viscosity to density without any force involvement. It can be obtained by dividing the viscosity of a fluid with its density [35].

$$v = \mu / \rho$$

Where
v = kinematic viscosity
μ = absolute or dynamic viscosity
ρ = density

Total Acid Number (TAN): 2.5g of oil was taken in a flask. 50 ml of methylated spirit was added to the flask, shake well and titrated against 0.1N KOH solution using phenolphthalein as indicator. Alkali was added till a pink color was established for a few seconds. The TAN was then calculated using the following formula [34].

$$Acid\,number=\frac{V\times N\times 56.1}{W}$$

Where as
V = volume of potassium hydroxide used
N = normality of Potassium hydroxide
W = weight in g of the sample

Iodine Value Determination: 0.2 g of respective oil was weighed into a conical flask. 10 ml of carbon tetrachloride and 20 ml of the Wij's solution were added to the flask and the solution was kept in dark for 30 min at room temperature. 15 ml of 10 per cent potassium iodide solution with 100 ml of distilled water were added to the flask. The resulting solution was titrated against 0.1 M sodium thiosulphate ($Na_2S_2O_3$), using starch as indicator till the end point where the blue black coloration becomes colorless. A blank titration was carried out at the same time starting with 10 ml carbon tetrachloride. Iodine value was then calculated by the following formula [36-40].

$$Iodine\,number = \frac{\{(B-S)\times N\times 12.69\}}{Weight\ of\ the\ sample}$$

Where
B = 0.1 N sodium thiosulfate required (ml) by blank
S = 0.1 N sodium thiosulfate required (ml) by sample
N = Normality of sodium thiosulfate solution.

Determination of the Saponification Value :2 g of each oil sample was weighted into a clean dried conical flask and 25 ml of alcoholic potassium hydroxide ($K(OH)_4$) was added. A reflux condenser was attached to the flask and heated for an hour with periodic shaking. The appearance of clear solution indicated the completion of saponification. Then 1 ml of 1 % phenolphthalein indicator was added and the hot excess alkali was titrated with 0.5 M hydrochloric acid (HCl) until it reached the end point where it turned colourless. A blank titration was carried out at the same time and under the same condition. The Saponification value was calculated as [34].

$$Saponification\,value = \frac{b-a}{m}\times 8.05$$

Where
b = 0.5 N HCl required (ml) by the blank
a = 0.5 N HCl required (ml) by the sample.

RESULT AND DISCUSSIONS

Physicochemical Analysis of Oil: Studies of various physicochemical characteristics identify the practical importance and provide bases for suitability and utility of various oils of plants origin in daily life. Physicochemical properties of oil like color, odor, density, specific gravity, refractive index, optical rotation, acid value, iodine value, saponification value etc indirectly tells about the quality of both essential and fixed oils.

In the present study essential oils obtained from the leaf of *S. laureola* (SVO) and *Z. armatum* (ZVO) and fixed oil from the fruit of *Z. armatum,* extracted with different solvent i.e*n*-hexane (ZHO) and petroleum ether (ZEO) were evaluated for physicochemical characteristics (Table 1). The essential oils were colorless to light apple white in appearance. The fixed oil i.e. (ZHO and ZEO) were brownish yellow and golden yellow respectively. [41, 42], both the volatile had pleasant odor while fixed oil had unpleasant odor. The percent yield of oils in the present study was 0.395±0.031, 0.198±0.018, 9.40±0.054 and 9.97±0.032 for SVO, ZVO, ZHO and ZEO respectively. Essential oil yield of SVO in the present study was less than the reported yield in Nepal which ranged from 0.93 to 1.12 %. The difference appeared, might be due to ecological factors. Similarly fixed oil showed variation in the yield, when extracted with different organic solvents.

Table 1: Physicochemical characteristics of S VO, ZVO, ZHO and ZEO. All Data are mean±SEM of three values.

S.No	Characteristics	SVO	ZVO	ZHO	ZEO
1	Colour	Colorless to light apple white	Colorless to light apple white	Brownish yellow	Golden yellow
2	Odour	Pleasant	Pleasant	Unpleasant	Unpleasant
3	% oil yield	0.395±0.031	0.198±0.018	9.40±0.054	9.97±0.032
4	Optical activity	+37.3°	+34.9°	+80.5°	+80.9°
5	Refractive index at 27°C	1.4194±0.0001	1.4180±0.0001	1.4176±0.0001	1.4171±0.0001
6	Specific gravity at 27°C	0.792±0.001	0.816±0.001	0.886±0.001	0.871±0.001
7	Carbon residue (%)	2.6633±0.051	2.8133±0.0051	2.2100±0.0033	2.3467±0.0051
8	Absolute viscosity	105.30±0.01	112.23±0.10	88.71±0.08	97.98±0.02
9	Kinematic viscosity	90.20±0.07	93.73±0.08	80.70±0.03	86.80±0.03
10	Total acid number (TAN) mg KOH/g	1.78±0.01	1.98±0.01	1.45±0.02	1.58±0.01
11	Iodine number	118±0.11	102±0.11	157±0.65	145±0.33
12	Saponification value (mg KOH/g oil)	109.00±0.67	105.33±0.84	163.33±1.02	151.67±0.69

Of the two volatile oil, it was found that SVO had a higher optical rotation (+37.3°) than the ZVO (+34.9°). The fixed oil extracted with n-hexane had a high optical activity as compared to the oil extracted with Petroleum ether. All the samples of fixed in the study have almost the same numerical values for refractive index ranged from 1.4171 to 1.4194 showing richness in long fatty acid chain in all samples.

Specific gravity is the ratio of the density of a respective substance to the density of water at 4°C [11]. Specific gravity values of oils are less than 1 for most of the oils except few containing oxygenated aromatic compounds [44]. In the present study, fixed oils from fruit of *Z. armatum* have high specific gravity values as compared to essential oils from the leaves of both plants. ZHO has a specific gravity of 0.886±0.001 followed by ZEO (0.871±0.001), ZVO (0.816±0.001) and SVO (0.792±0.001). These findings were found well in lineage with the values determined for seed oils of some plants from Congo [45]. Our results are little inconsistent with Elert (2000), who described that most of the oils are characterized with specific gravity ranges from 0.9100 to 0.9400. The number of milligram of KOH, which are required to neutralize the free fatty acids present in one gram of oil. The acid number measures the amount of acids present in an oil [46]. Acid value is an indirect method for determination of free fatty acid of amount in oil samples and its edibility [46]. Oil with low free fatty acids has more significant usage [47]. The Total acid number (TAN) values recorded in the present study were 1.78±0.01, 1.98±0.01, 1.45±0.02 and 1.58±0.01 for SVO, ZVO, ZHO and ZEO respectively. These values were found in the permissible limits i.e. 10 mg KOH/g of oil and found to be suitable for dietary purposes, as they contain lower fatty acid contents [48]. Values obtained in the present study were found lower than seed oil of *Jatrophacurcas*[46] and seeds oil of five Nigerian species [49].

Carbon residue values for SVO were found 2.65%, less than for ZVO (2.80 %). For fixed oils these values werer found smaller than essential i.e. 2.21% for ZHO and 2.35% for ZEO. Absolute viscosity was found quite higher for SVO in all samples. Similarly other values related to viscosity like viscosity index and kinematic viscosity were also calculated showing variant results (Table 1). Similar values were also determined for different *Citrus* seed oils [52] and *Jatrophacurcas* oil [50], which strengthened the present study.The number of milligram of KOH, which are required for the complete saponification of one gram of oil is called saponification value. Saponification values determined were found greater for fixed oil of *Z. armatum* fruit as compared to SVO and ZVO. The oil samples have saponification values of 109.00±0.67, 105.33±0.84, 163.33±1.02 and 151.67±0.69 mg/KOH/g for SVO, ZVO, ZHO and ZEO respectively. The saponification values for ZHO and ZEO are more or less in lineage with the work done by previous workers like [45] and Akinyeye *et al.* [52]. Essential oils saponification values were found in accordance with that of Ejikeme *et al.*, [52], thus strengthening our findings. All the oil samples are found in the range reported for plants (lower than the 188-196) but not useful in soup industry as these values are much lesser than the required value (±300) [54]. Iodine value is a Number of grams of iodine absorbed per 100 gram of fat or oil is known as Iodine value. It is the measure of unsaturation in oil. The iodine value is the indicative of fats and oils unsaturation. Fats and oils with higher unsaturation show high iodinevalue [37, 38]. The iodine valve observed for the oil samples in the present study were 118±0.11, 102±0.11, 157±0.65 and 145±0.33 for SVO, ZVO, ZHO and ZEO respectively. These values were found in the permissible range for semi-drying of oil (100-300) [39]. Recorded data for iodine number showed that all the oil samples have high unsaturated fatty acids contents [50]. The present study also showed inconsistency with that of Essein Amadi (2009) [48], Mohanty *et al.* (2010) and Akinyeye *et al.* (2011), who studied various oil samples of plant origin and reported smaller iodine value for these oils.

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