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RESEARCH ARTICLE

Proximate analysis, physicochemical properties and chemical characterization of *Rosmarinus officinalis* L. oil

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Abstract

Rosmarinus officinalis L. is a herb belonging to the family Lamiaceae that originates from the Mediterranean region, a common medicinal and aromatic plant, grown in many parts of the world including Ethiopia. The dried leaves of *Rosmarinus officinalis* cultivated in Ankober woreda, Ethiopia were subjected to hydro distillation for 3 h using a Clevenger-type apparatus. The percent yield (1.23 %), physico-chemical properties such as refractive index (1.463), relative density (0.899), optical rotation (-5o), solubility, colour and odour were determined. Proximate analysis of *Rosmarinus officinalis* leaves such as total ash, water soluble ash, acid insoluble ash, moisture content, pH, were found to be (7.6), (3.6), (1.7), (10.4) and (5.9) respectively. The essential oil of *Rosmarinus officinalis* were analysed using GC-MS and the main components of the *Rosmarinus officinalis* oils are α -pinene (50.8 %), 1, 8-cineole (24.4 %), camphene (5.2 %), camphor (3.8 %), caryophyllene (2.9 %) and β-pinene (2.1 %).

Keywords: *Rosmarinus officinali*s, Essential oil**,** GC/MS, Proximate analysis

Introduction

Medicinal and aromatic plants are receiving considerable attention all over the world because of their vast untapped economic potential, especially in the use of herbal medicines. Aromatic and medicinal plants occupy an important place in the socio-cultural, spiritual and health care aspects in Ethiopia. Ethiopia possesses a wide range of potentially useful medicinal plants including *Rosmarinus officinalis* L. (Mekonnen & Manahlie, 2017).

Rosmarinus officinalis (Rosemary) is among the essential oil-bearing plants, which are widely used in fragrance, cosmetics, food and pharmaceutical industry. In Ethiopia, *Rosmarinus officinalis* locally known as "Yetibs Qitel" is used as a flavor of varieties of dishes and spices ingredients. Rosemary essential oil is also used for aromatherapy, cosmetics, flavouring and preservation of food products (Melka et al., 2018).

Rosmarinus officinalis, a herb belonging to the family Lamiaceae that originates from the Mediterranean region is a common medicinal and aromatic plant, grown in many parts of the world. Traditionally *Rosmarinus officinalis* leaves are used as a spice in foods and beverages as well as herbal medicine for various spasmodic conditions such as renal and biliary colic (Al-Mukhtar et al., 2013).

Rosmarinus officinalis essential oil is important for its medicinal uses and its powerful antibacterial, cytotoxic, antimutagenic, antioxidant, antiphlogistic and chemo-preventive properties. Biological activities of the essential oils/extracts are correlated to the presence of specific chemical compounds (Hussain et al., 2010).

Rosemary essential oil can be obtained from stems, leaves, and flower twigs. Essential oil is dominated by 1,8-cineole, α-pinene, camphene, α-terpineol, and borneol as principal constituents (Banjaw et al., 2016; Habtemariam, 2016). Another group of promising secondary plant metabolites found in *Rosmarinus officinalis* L. are triterpenes, flavonoids, phenolic and diterpenes (Borrás-Linares et al., 2014).

The composition pattern of the *Rosmarinus officinalis* L., concentration of individual components, and yield depend on many intrinsic and extrinsic factors. External factors include environmental factors (climate and habitat conditions, sowing date, and fertilizer variations), cultivation conditions (agro techniques) (temperature, humidity, radiation, wind, soil properties, geographical location, and harvest time and methods), and postharvest techniques (drying methods and extraction, distillation time, quantification methods, and conditions of analysis) (Moghaddam & Mehdizadeh, 2017).

Rosmarinus officinalis is rich source of different bioactive compounds and Ethiopia has different types of climates, soil types and geographical locations. For this reason, the objective of this work was to study the composition of essential oils and proximate analysis of the plant harvested in Ankober woreda, Ethiopia (elevation of about 2,465m from sea-level).

Materials and Methods

Plant material

Rosemary leaves were collected from Ankober woreda in a single collection site in May, 2016. The leaves were air-dried under the shade at the ambient temperature, protected from the direct sun light. The identity of the plant specimen was confirmed by Botanist at the Department of biology of Debre berhan University and the voucher Number was Ank.110.

Extraction of essential oils

Extraction of essential oil from leaves was carried out by a hydro-distillation. Dried leaves (100 g) were weighed and hydro distilled for three hours using Clevenger-type glass apparatus. The oil sample obtained from hydro distillation was freed from water by adding anhydrous sodium sulfate and stored in sealed vials at +4°C until GC-MS analysis.

Chemical composition identification

Determination of the chemical composition of the extracted essential oils from *Rosmarinus officinalis* were carried out by Gas Chromatography-Mass Spectroscopy (GC/MS). GC/MS analysis was performed with HP5890 series II coupled with mass spectrometry HP5972 series detector and an electron ionization system, equipped with a capillary column HP5 (30 m, 0.53 mm, 0.25 µm films). The carrier gas was helium, with a gas flow of (0.5 mL/min). Oven temperature was kept at 50 °C for 4 minutes and programmed to 280 °C at a rate of 3°C/minutes. Injector temperature was 250 °C and the detector temperature was 280 °C. Mass spectra were taken at 70 eV.

Proximate analysis

The powder samples of rosemary leaves were subjected to evaluate its moisture content, pH, total ash, water soluble ash and acid insoluble ash.

Moisture content

A mass of 1 g of the powdered plant material was spread in a thin layer in the crucible of the moisture balance apparatus. Temperature was set at 100° C. Plant material was kept under this temperature until the moisture content attained a constant value (Koleilat et al., 2017). The amount of oil was subtracted from the final product.

Determination of pH Levels

1.0 g of powdered herbal leaf was measured by an electrical balance and then transferred into 25 ml conical flask; 25 ml of distilled water was added and heated on hot plate to boiling and left to cool down. The aqueous herbal extracts were filtered into 25 ml volumetric flask and filled by distilled water to the mark and was determined by using a calibrated pH meter (Moses et al., 2013).

Total ash, water soluble ash, acid-insoluble ash

Total ash, water soluble ash and acid-insoluble ash was carryout according to World Health Organization (Quality control methods for herbal materials, 2011)

Physico-chemical analysis

Physicochemical properties are useful methods in determining the quality of essential oils. The Physico-Chemical properties (refractive index, relative density, optical rotation, Solubility, color and odour) were determined according to standard analytical methods recommended by (AOAC 200)(Horwitz, 2000; Koleilat et al., 2017).

Results and Discussion

The percentage yields of essential oil obtained from the hydro distillation of the leaves of *Rosmarinus officinalis* was 1.23 % of (v/w, based on the dried leaves).

The leaf sample of *Rosmarinus officinalis* and essential oils were tested for relevant proximate and physicochemical parameters and the results are presented in (Tables 1&2). From the result it was found that among the characteristic properties, the relative density, Optical rotation, Refractive index, colour and odour of the oil were good agreement with the reported result in British Pharmacopoeia.

Table 1. Proximate analysis of *Rosmarinus officinalis* L. leaves

Tests	values
Total ash	7.6
Water soluble ash	3.6
Acid insoluble ash	1.7
Moisture content	10.4
pH	5.9

Table 2. Physico-Chemical characteristics of *Rosmarinus officinalis* L. essential oils in comparison with British Pharmacopoeia

Physico-Chemical Characteristics	Value	British Pharmacopoeia (2008)
Relative density	0.899	0.895 to 0.920
Refractive index	1.463	1.464 to 1.473
Optical rotation	-5^{o}	-5 to 8
Appearance	Colourless liquid	Colourless to pale yellow liquid
Odour	Characteristic	Characteristic
Solubility	Soluble in alcohol	------
Color	Colourless	Colourless to pale yellow

"----"not identified

The chemical composition of rosemary essential oil is shown in Table 3. As shown in the table, a total of seventeen volatile components, representing 99.99 % of the total composition, were identified from the dried rosemary leaves. The main components were α-pinene (50.8 %), 1,8-cineole (24.4 %), camphene (5.2 %), camphor (3.8 %), β-caryophyllene (2.9 %) and β-pinene (2.1 %).

α-pinene, camphene, β-pinene, β-myrcene, limonene, 1,8-cineole, α-terpineol, camphor, borneol, bornyl acetate and verbenone are the marker components present in the oil. All the marker components were detected in the oil extracted from the leaves of *Rosmarinus officinalis* obtained from Ankober Woreda.

Table 3. Percentage composition of the volatile oils isolated from *Rosmarinus officinalis* leaves.

Compound	RI	Percentage (%)
α-pinene	924	50.8
camphene	939	5.2
β-pinene	968	2.1
β-myrcene	982	0.7
α-terpinene	1008	0.4
limonene	1029	1.7
1,8-cineole	1023	24.4
ϒ-terpinene	1050	0.7
α-terpinolene	1078	0.5
linalool	1092	1.3
camphor	1138	3.8
borneol	1160	1.5
α-terpineol	1184	0.8
verbenone	1203	0.5
bornyl acetate	1279	1.6
β-caryophyllene	1417	2.9
α-humulene	1451	0.4

RI- Retention Indice

Comparison of the obtained results with the literature data, percentage composition of α-pinene is greater than the values reported from different countries (Gohar et al., 2009). The percentage camphene is in a good agreement with oils of Cuba, Argentina and Italy origin. The percentage content of β-pinene is in a good agreement with Iran, but less than the values reported in Morocco, Spain, Frances, Algeria Cuba, and Argentina, but greater than values reported in Italy. The percentage of 1, 8-cineole is less than Morocco and Algeria but greater than Cuba, Argentina, Italy, Spain, France and Iran.

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