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Determinations of Physicochemical Properties, Proximate Compositions, and Characterization of Chemical Constituents of *Thymus schimperi* Essential oil

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Abstract

Thymus schimperi is endemic to Ethiopia and it is recognized in central, eastern and northern Ethiopia. Investigation has been carried out to determine the physicochemical properties, and to analyze the proximate values of the *T. schimperi* leaves and chemical composition of its essential oil grown at Ankober district, Ethiopia. The shade dried ground leaves of *T. schimperi* were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus to get the leaf essential oil (1.85%). The physicochemical properties of the essential oil such as refractive index (1.484), relative density (0.941), optical rotation (-1.8^{o}), solubility (water insoluble and ethanol soluble), color (yellowish) and odor (aromatic) were determined. Proximate values of *T. schimperi* leaves such as total ash, water soluble ash; acid insoluble ash, moisture content and pH were analyzed to be 9.14, 3.34, 2.52, 12.4 and 6.17, respectively. In the GC-MS analysis of the essential oil of *T. schimperi* a total of thirteen volatile components, representing 98.82 % of the total, were identified. The main components of the essential oil are thymol (71.02 %), p-myrcene (15.25 %), ϒ-terpinene (9.02 %), phenylethanone (1.33 %) and α-phellandrene (1.062 %).

Keywords*:* Thymus schimperi, essential oil, physicochemical properties, proximate analysis, GC-MS

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Introduction

Aromatic and medicinal plants occupy an important place in the socio-cultural, spiritual and health care aspects in Ethiopia (Asfaw *et al*, 2000). Ethiopia possesses a wide range of potentially useful medicinal plants including *Thymus schimperi* (locally known as 'Tossign' (Amh.)). The genus *Thymus* includes about 350 species worldwide largely distributed in temperate zones and it is uncommon in the African tropics (Mekonnen *et al*, 2017). Ethiopia has considerably abundant Lamiaceae herbs growing at different areas including its wild growing species

(Hailemariam *et al*, 2013). The wild growing species of thyme, *T. schimperi*, is endemic to Ethiopia, and comparatively well-known in central, eastern and northern Ethiopia. *T. schimperi* is growing on edges of roads, in open grassland, on bare rocks and on slopes, between 2200-4000 m altitudes (Hailemariam *et al*, 2013; Haji *et al*, 2016).

The genus *Thymus* is known for its medicinal value. In Ethiopia folk medicine the thyme plant species are used in a variety of forms, fresh or dried, for treating headache, cough, stomachache, earache, liver disease and gonorrhea, and for flavoring purposes. Specifically, *T. schimperi* is used to flavor hot beverages like tea, coffee, and different kinds of stew (Mekonnen *et al*, 2017). The medicinal value of the different species of this genus is related to their chemical composition. Reportedly, the primary components of the leaf essential oil of *T. schimperi* are thymol and carvacrol (Mekonnen *et al*, 2017; Damtie *et al*, 2017; Hamid *et al*, 2011).

As far as we know, physicochemical properties of *T. schimperi* leaf essential oil and proximate value of the leaf has not been described yet in Ethiopia. Therefore, this investigation has been carried out to determine the physicochemical features of the essential oil, proximate values of the leaves, and in the course to identify the marker chemical compounds of the leaf volatile oil of *T. schimperi* grown at Ankober district.

Materials and Methods

Collection and preparation of plant material

Fresh leaves of *T. schimperi* were collected from Ankober district and air-dried in the shade to protect it from direct sun light. The dried leaves were stored in paper bags until used for hydro-distillation and in turn proximate analysis. The identity of the plant specimen was confirmed by a professional botanist, Department of Biology, Debre Berhan University.

Extraction of essential oils

The ground *T. schimperi* leaves (100 g) were extracted by hydro-distillation using Clevenger like apparatus (3 h) for their essential oil. The resulting oil was dried by adding anhydrous sodium sulfate, and the oil was stored in sealed vials at 4 ^{o}C for further analysis.

Proximate analysis

The ground *T. schimperi* leaves were subjected to determination of the moisture content, pH, total ash, water soluble ash and acid insoluble ash following known protocols underneath.

*Moisture content***:** A mass of 1 g of the powdered plant material was spread in a thin layer in the crucible and placed in a digital oven (105° C) until a constant mass attained (Koleilat *et al*, 2017).

*Determination of pH***:** One gram of ground material in conical flask with 25 mL of distilled water was heated on hot plate to boiling, and left to cool down. The resulting aqueous herbal extract was filtered into volumetric flask which was filled by distilled water to the mark for pH measurement (Maobe *et al*, 2013).

*Total ash, water soluble ash, and acid insoluble ash***:** Determination of total ash, water soluble ash and acid insoluble ash was carried out according to World Health Organization Quality control methods for herbal materials (Maobe *et al*, 2013; WHO,2011).

Physicochemical Properties

Physicochemical properties are useful methods in determining the quality of essential oils. Accordingly, refractive index, relative density, optical rotation, solubility, color and odor were determined by the recommended Standard Analytical Methods reported elsewhere (Feldsine *et al*, 2002 Koleilat *et al*, 2017; Horwitz *et al*, 1925).

Marker Compound Identification

Chemical analysis of the sample was made by Gas-Chromatography (HP5890) coupled to Mass Spectroscopy (HP5972) (GC-MS). An electron ionization system, equipped with a capillary column HP5 (30 m, 0.53 mm, 0.25 μm films) was used. The carrier gas was helium, with a gas flow (0.5 mL/min). Oven temperature was kept at 50 °C for 4 min and programmed to 280 °C at a rate of 3 °C/min. Injector and detector temperatures were 250 °C and 280 °C, respectively. Mass spectra were taken at 70eV. The identification of the components separated by GC-MS was made by comparing the obtained mass spectra for each component with the values stored in mass spectra library Wiley 8.

Results and Discussion

The essential oil yield obtained by hydrodistillation of *T. schimperi* leaves (1.85% (w/w, dry basis)) was higher than the reported one (1.02-1.48%) (Mekonnen *et al*, 2017; Haji *et al*, 2016). The results of the physicochemical properties (Table 1) and proximate determinations of the leaves (Table 2) are presented below.

Table 1. Physicochemical characteristics of *T. schimperi* EO

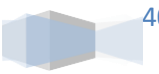
Attribute	Values
Relative density	0.941
Refractive index	1.484
Optical rotation	-1.8
Appearance/ color	Yellowish liquid
Odour	Aromatic
Solubility	Soluble in ethanol
Relative density	0.941

Table 2. Proximate analysis of *T. schimperi* leaf

Attribute	Values
Total ash	9.14 %
Water soluble ash	3.34 g
Acid insoluble ash	2.52 g
Moisture content	12.4 %
PH	6.17

Based on the physicochemical and proximate analysis, the characteristic properties of the essential oil like odor, color, relative density, optical rotation, solubility and refractive index were more or less in good agreement with the reported values (Horwitz, 1925; British Pharmacopeia, 2010).

The chemical composition of *T. schimperi* essential oil as done by GC-MS (Table 3) showed a total of thirteen volatile compounds representing 98.82 % of the total composition were identified from the essential oil.



The marker components of the leaves essential oil are thymol (71.024 %), p-myrcene (15.250 %), ϒ-terpinene (9.020 %), phenylethanone (1.326 %) and α-phellandrene (1.062 %).

Table 3. Chemical composition of *Thymus Schimperi leaves essential* oil

Compound	RT	% composition
α-phellandrene	10.893	1.062
α-Pinene	11.116	0.213
p-Myrcene	16.570	15.250
ϒ-Terpinene	18.219	9.020
Sabinene hydrate	18.336	0.046
Ocimene	118.991	0.051
Linalool	19.655	0.159
Carvacrol	33.260	0.104
Thymol	33.823	71.024
Phenylethanone	34.498	1.326
1,2- Benzenediol	36.720	0.374
β-Sesquiphellene xide	39.558	0.108
Caryophyllene oxide	42.038	0.087

RT=Retention Time

Conclusion

In this study, physicochemical attributes of essential oil of *T. schimperi* leaf grown in Ankober district, proximate properties of its leaf and chemical composition of the essential oil were analyzed. The GC-MS analysis showed that thymol (71.024%) is the major constituent which is an important component of antimicrobial agents. This work also provides useful information for the future applications of the leaf/essential oil.

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Conflict of interest

The authors declare that there is no conflict of interest.

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