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Chemical constituents, physicochemical properties and antibacterial activity of leaves essential oil of *Ocimum urticifolium*

Ketema Alemayehu*, Mathewos Anza**, Destaw Engdaw, Abedelfeta Mohammed

College of Natural and Computational Science, Department of Chemistry, Wolaita Sodo University, P. O. Box 138, Wolaita Sodo, Ethiopia

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ABSTRACT

Objective: To determine chemical compositions, physicochemical properties and evaluating antibacterial activities of essential oils extracted from leaves of *Ocimum urticifolium* (*O. urticifolium*).**Methods:** Essential oil of *O. urticifolium* was extracted by hydrodistillation technique. A number of phytochemical screening tests were applied to identify the classes of compounds in the leaves extract of *O. urticifolium*. Gas chromatography and gas chromatography/mass spectrometry were used to characterize the chemical components in the essential oil. The agar diffusion method was used to evaluate the antibacterial activity as per of standard procedure.**Results:** Phytochemical screening of crude extract revealed that the presence of tannins, glycosides, saponins, flavonoids, steroids, terpenoids and phenols. The obtained oil yield is $(0.33 \pm 0.11) \%$ (v/w). Analysis of oil using gas chromatography and gas chromatography/mass spectrometry showed a total of 22 components, the abundance of monoterpene and sesquiterpenes (98.99%). The percentage composition of monoterpene in the oil was α -pinene (22.105%), eugenol (21.099%), while sesquiterpenes α -cubebene (11.341%), α -bisabolene (9.945%), α -caryophyllene (7.709%), α -caryophyllene oxide (5.754%), and copaene (3.594%). The oil inhibited the growth of *Staphylococcus aureus* and *Escherichia coli*, while no activity was shown to *Salmonella typhi*.**Conclusions:** The *O. urticifolium* is a rich source of various classes of chemical constituents and the antibacterial activity of the oil could be attributed mainly to these compounds.

1. Introduction

Medicinal plants have played a pivotal role in the primary healthcare and known to provide a rich source of raw materials for natural products as well as used as a traditional treatment for numerous human and livestock ailments for thousand years in Africa, Asia and other parts of developing world. Particularly those living in rural areas of the developing countries, it is continued to be used as the primary source of medicine[1]. Essential oils are a diverse group of natural products that are important sources of aromatic and flavoring chemicals in food, industrial, and pharmaceutical products. Essential

oils are largely composed of terpenes and aromatic polypropanoid compounds derived from the acetate-mevalonic acid and the shikimic acid pathways, respectively. The compositions of essential oil in plants are varies due to genetic and environmental factors that influence genetic expression[2]. The contents of essential oil in plant tissue are also varies with developmental stage[3] and can be varied by extraction methods[4].

Among the plants known for medicinal value, genus *Ocimum* belongs to the family Lamiaceae, is very important for their therapeutic potentials. A genus *Ocimum* comprises more than 150 species, which are distributed from tropical to subtropical regions[5]. The species *Ocimum urticifolium* (*O. urticifolium*) Roth is an erect herbaceous annual plant, or sometimes grown as a short-lived perennial in some areas. It grows into a bushy shape up to about 3 m tall and some varieties may even grow taller. The stems are herbaceous in young tissue. However, these become woody as the plant matures. The leaves are broad, soft, oval shaped and 5 to 8 cm long. The leaves are green to bright green or red coloured and larger or smaller, depending on the

*Corresponding author: Ketema Alemayehu, College of Natural and Computational Science, Department of Chemistry, Wolaita Sodo University, P. O. Box 138, Wolaita Sodo, Ethiopia.

Tel: +25128541651

E-mail: city2854@gmail.com

Mathewos Anza, College of Natural and Computational Science, Department of Chemistry, Wolaita Sodo University, P. O. Box 138, Wolaita Sodo, Ethiopia.

Tel: +251913348379

E-mail: mathewosanza@gmail.com

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form and variety, and on the fertility of the soil.

In the different locality of Ethiopia, an indigenous person uses *O. urticifolium* for various disease treatments[6]. The vernacular name of *O. urticifolium* is “Guluuwa/Desha-dunkiya (Dawro)”, “Damakase (Amharic)”, of the language of Ethiopia. The present study aims to determine chemical compositions, physico-chemical properties and evaluate antibacterial activities of essential oils extracted from leaves of *O. urticifolium*.

2. Materials and methods

2.1. General experimental materials

Gas chromatography-mass spectrometer (GC-MS), Abbe's refractometer, Polar meter (ADP 220 polarimeter, Bellingham + Stanley Limited Model No. 36-220, England). And all chemicals and reagents in this study were used pure analytical grade level Indian and USA products.

2.2. Plant material collection

Fresh leaves of *O. urticifolium* were collected from different location of Mekelle city 780 km from Addis Ababa, the capital of Ethiopia, in March 2013. The plant material was identified in Addis Ababa University National Herbarium Center, and voucher specimens were deposited at the institute with voucher number 81857. The leaves were washed thoroughly with water and air dried in shadow for 7 days and preserved until further used.

2.3. Extraction of essential oil

Air dried leaves of *O. urticifolium* (250 g) in 2500 mL distilled water were loaded in round bottom flask and subjected to hydro distillation in a Clevenger apparatus for five hours at 96 °C to get essential oil. On heating the flask essential oil glands present in the plant material get ruptured. The steam and essential oil vapours generated in the flask pass through a condenser to remove the heat which finally converts the vapours in to liquid. The condensate (mixture of essential oil and water) was collected in separatory funnel. Since, the water and essential oil have different densities, essential oil floats on the surface of the water in the separators funnel. The essential oil was separated from aqueous layer using a 100 mL capacity separatory funnel. The extract was transferred in to an amber glass, and kept in the refrigerator until further analysis.

2.4. GC/MS analysis

An Agilent model 6890 GC interfaced to a 5975 mass selective

detector was used for mass spectral identification of the components of the oils. A fused silica column 5% vinyl-poly-dimethyl-siloxane (DB-5MS 30.00 m × 0.25 mm × 0.25 micron film thickness) was used. The oven temperature was initially maintained at 70 °C then programmed to 120 °C at the rate of 5 °C/min and to 280 °C at the rate of 10 °C/min with final hold time of 30 min. The carrier gas was helium, at a flow rate of 1.5 mL/min, and the injection volume 0.2 µL neat with splitting ratio 90:1 and sampling rate is 2. In mass spectrometry, electron-impact ionization was performed in electron energy of 70 eV. The MS temperature was 230 °C (source), 150 °C (Quad). Data acquisition was performed with mass selective detector consultation D.02.00.275 with National Institute Of Standards And Technology Library Ver. 2.0 d software for the scan ranges 50–1 000 amu. The identification of compounds was also based on the Kovats retention indices and retention time.

2.5. Phytochemical screening

Chemical tests were carried out on the solvent extract or the powdered specimens of the leaves and the essential oil of *O. urticifolium* using standard techniques for the detection of sterols, saponins, phenolics, tannins, flavonoids, terpenoids, alkaloids, anthraquinone, and cardiac glycosides[7,8].

2.6. Physicochemical properties of oil

The physicochemical properties are a significant parameter to assess the quality of the oil and thus can be used as basic criteria for its identification. Therefore in the present study, chemical and physical characteristics of the essential oil of the leaves of *O. urticifolium* were measured. The physicochemical properties of the oil determined were color, odor, % yield, density, optical activity, refractive index, specific gravity, total acid number and saponification value. The experiments were carried out in triplicate and the data were reported as mean ± SD.

2.6.1. Color determination

Color determination is one of a physical parameter of essential oils. Thus, in the present study the color of essential oil was determined by observing physical appearance of the extracted oils from *O. urticifolium* in day light.

2.6.2. Odor determination

Odor of the essential oils was determined by organoleptic evaluation following Trease and Evans[9]. Organoleptic testing is a process of using our sense to test essential oil. Thus, in the present study, smell strips were used with one drop of essential oil on them, and then began smelling the essential oil which extracted from *O. urticifolium* at different time interval.

2.6.3. Determination of optical rotation

The optical rotation of essential oil was determined as per of standard procedure. About 10 mL Polari meter tube containing essential oil was placed in the trough of the instrument between polarizer and analyzer. 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 clockwise from the zero position to obtain the final reading, the rotation is levo (-) if clockwise and dextro (+) if anti clockwise[10].

2.6.4. Determination of specific gravity

Specific gravity of the essential oil of *O. urticifolium* was measured by using the standard procedure[10]. A clean 50 mL specific gravity bottle was weighted (W0). 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 (W1). Same process was repeated, but using oil samples instead of water and weighted again (W2). The specific gravity of the oil samples were calculated using (equation 1).

$$\text{Specific gravity of test sample} = \frac{W_2 - W_0}{W_1 - W_0} \text{ ----- (Equation 1)}$$

where, W0 = weight of empty specific gravity bottle; W1 = weight of water + specific gravity bottle; W2 = weight of test sample + specific gravity bottle.

2.6.5. Determination of refractive index

The refractive index of essential oil of *O. urticifolium* sample was determined by using Abbe refractometer as per of procedure[11]. Abbe refractometer was placed on the table, and then the prism was cleaned gently with cotton. Two drops of the oil was put on the prism and the prisms were clamped together firmly. The light source was fixed so that the light was reflected through the prisms and the instrument adjusted until the borderline between the light and dark halves of the field of view exactly coincides with the cross hairs of the telescope. The mirror was adjusted in such a way that maximum light enters the window prism. Then the prim was rotated with the help of moveable arm until the border line appears in the field. The boundary line was adjusted for sharp and the refractive index reading was noted at 25 °C.

2.6.6. Determination of pH value

About 2 mL of the oil was poured into a clean dry 25 mL beaker. The electrode was first cleaned with cotton and then immersed into the sample and the pH value was read and recorded at (25 °C).

2.6.7. Solubility

The solubility of the volatile oil was determined by mixing increment volumes of the volatile oil in specified volumes of the

following solvents: water, chloroform, and alcohol.

2.6.8. Determination of density

About 1 mL of the oil was taken and after the weight was measured, then density was calculated as ratio of to its volume.

2.6.9. Determination of acid value

About 25 mL of petroleum ether and 25 mL ethanol were mixed in a 150 mL beaker. The resulting mixture was added to 1.5 g of oil in a 250 mL conical flask and 3 drops of phenolphthalein was added to the mixture. The mixture then was titrated with 0.1 mol/L KOH to the end point with consistent shaking until a pink color was observed and the volume of 0.1 mol/L KOH (V) was noted[12]. It was calculated by using (equation 2).

$$\text{Acid value} = \frac{56.1 \times N \times V}{M} \text{ ----- (Equation 2)}$$

where: N = molarity of KOH; M = mass of the oil used; V = volume of 0.1 mol/L KOH used for titration.

2.6.10. Determinations of saponification value

Saponification value of essential oil of *O. urticifolium* sample was determined as per of standard procedure[12]. About 1.5 g of the oil sample was added to a flask with 25 mL of 0.5 mol/L ethanolic KOH and then attached to a condenser for 30 min to ensure the sample is fully dissolved. After sample has cooled 1 mL of phenolphthalein was added and titrated with 0.5 mol/L HCl until a pink endpoint has reached. Then the saponification value calculated by using (Equation 3).

$$\text{Saponification value} = \frac{(S-B) \times M \times 56.1}{\text{Sample weight}} \text{ ----- (Equation 3)}$$

where, S = sample titre value; B = blank titre value; M = molarity of the HCl; 56.1 = molecular weight of KOH.

2.7. Evaluation of antibacterial activity of the essential oil

The agar diffusion method was used to evaluate the antibacterial activity as per of standard procedure[13]. Clinical isolated pathogenic microorganisms [*Salmonella typhi* (*S. typhi*), *Escherichia coli* (*E. coli*), and *Staphylococcus aureus* (*S. aureus*)] were obtained from the microbiology laboratory of college of veterinary medicine of Mekelle University, Ethiopia. The stocks were maintained on nutrient agar slant and sub-culture in nutrient broth for incubation at 37 °C prior to each antibacterial test. Inoculation of the test organisms on nutrient agar prepared plates was achieved by flaming a wire loop on a spirit lamp, cooling the wire loop (air cooling) and fetching the test organisms. The inoculum suspension was streaked over the surface of the media using sterile cotton swab to ensure confluent growth of the organisms and the turbidity was adjusted

to the standard inoculum of Mac Farland scale 0.5 [$\sim 10^6$ CFU/mL]. A total of 5 mm diameter discs were prepared with Whatmann No.1 paper and used for the study, and putting in vials-bottles and sterilizing in an oven at 150 °C for 15 min. Prepared discs containing the essential oil and positive controls were carefully placed on the inoculated plates using a sterilized forceps in each case. The plates were then turned upside-down and inoculate at 37 °C for 24 h in an incubator. After incubation, the inoculated plates were measured for zones of inhibition (in mm diameter by using electronic digital caliper). Penicillin (10 IU/mL), and tetracycline (30 µg/mL), were used as positive controls, positive control discs were tested on the same microorganisms under the same conditions.

2.8. Statistical analysis

All data are presented as the mean \pm SD of three measurements. The comparisons between the control groups (penicillin and tetracycline) and the test group (antibacterial activity of essential oil of *O. urticifolium*) were performed by SPSS version 15. Statistical significance *P*-level was selected as 0.05.

3. Results

3.1. Preliminary phytochemical screening

The successive crude extracts of leaves of *O. urticifolium* revealed that the presence of various bioactive components, such as tannins, glycosides, saponins, flavonoids, steroids, terpenoids, phenols and the absence of anthraquinones whereas the essential oil contains only terpenoids and phenols (Table 1).

Table 1
Phytochemical screening of crude extracts and essential oil of *O. urticifolium*.

Bioactive compounds	Crude extract	Essential oil
Tannins	+	-
Glycosides	+	-
Saponins	+	-
Flavonoids	+	-
Steroids	+	-
Anthraquinone	-	-
Terpenoids	+	+
Phenols	+	+

+: Positive; -: Negative.

3.2. Physicochemical properties

As indicated in Table 2, the yield of essential oil content observed in leaves of *O. urticifolium* was (0.33 ± 0.11) % (v/w), light yellow color and the volatile oil had pleasant odor. The volatile oil was miscible in organic solvents like ethanol and partial soluble in chloroform, it is immiscible in water. The specific gravity, optical

activity and refractive index were also determined. The specific gravity was obtained to be 0.920 ± 0.125 which is close to pumpkin seed oil of 0.918[14]. The acid value recorded in the present study was 14.137 ± 2.133 . The saponification value obtained for the oil samples was (181.020 ± 1.112) mg KOH/g for *O. urticifolium*.

Table 2
Physicochemical properties of the oil.

Parameters	Results
Colour	Light yellow
Odour	Pleasant
Oil yield [% (v/w)]	0.330 ± 0.110
Specific gravity (25 °C)	0.920 ± 0.125
Optical rotation (25 °C)	-14.000 ± 0.063
Refractive index (25 °C)	1.502 ± 0.521
pH (25 °C)	8.600 ± 0.020
Ethanol	Soluble
Chloroform	Partially soluble
Water	Insoluble
Density (g/mL)	0.870 ± 0.151
Acid value	14.137 ± 2.133
Saponification value	181.020 ± 1.112

The results were presented as mean \pm SD, *n* = 3.

3.3. Chemical compositions

The gas chromatogram result of essential oils of *O. urticifolium* was revealed that total of 22 compounds which represents more than 98.999%. The major constituents of the essential oil of were analyzed by GC-MS. The results along with mode of identification of individual components of volatile compounds identified in the essential oils of *O. urticifolium* in order of elution from column with their respective percentage composition in each species (Table 3).

Table 3
Chemical compositions of *O. urticifolium* essential oil.

Peak No.	Rt (min)	Compound identified	RI	Composition (%)
1	5.386	α -Pinene	948	22.105
2	5.544	3-Carene	950	1.348
3	6.618	Linalylanthranilate	2157	0.836
4	7.280	2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	993	0.360
5	7.333	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	966	0.942
6	9.185	Trans-3-Carene-2-ol	1136	0.326
7	12.678	Eugenol	1392	21.099
8	12.962	Copaene	1221	3.594
19	13.131	α -Bourbonene	1339	1.452
10	13.762	α -Caryophyllene	1494	7.709
11	13.941	α -Bergamotene	1430	1.532
12	14.288	1,4,7-,Cycloundecatriene,1,5,9,9-tetramethyl-,Z,Z,Z-	1579	1.346
13	14.751	α -Cubebene	1339	13.087
14	14.919	α -Guaiane	1523	0.520
15	14.951	ϵ -Gurjunene	1461	0.527
16	15.109	α -Bisabolene	1500	9.945
17	15.319	(+)- δ -Cadinene	1469	1.445
18	15.772	Longipinocarveol, trans-	1599	0.550
19	16.150	(-)-Spathulenol	1536	0.510
20	16.235	Caryophyllene oxide	1507	5.754
21	16.561	Ledene oxide-(II)	1293	1.166
22	17.118	6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol	1690	2.846
Total	98.999			

RT = Retention time; RI = Retention index.

It was found to be a mixture of monoterpenoids (45.834%) and sesquiterpenoids (51.983%) (Table 4). α -Pinene was the major monoterpene constituents of oil of leaves of *O. urticifolium* (22.105%), $C_{10}H_{16}$ (136), with fragment ions at 136, 121, 105, 77, 67, 53 at retention time 5.386 with base peak 93. At retention time 12.678 should eugenol (21.099%), $C_{10}H_{12}O_2$, fragment ions at 164, 149, 137, 131, 121, 103, 91, 77, 55 with base peak 164. Similarly sesquiterpenes ($C_{15}H_{24}$) viz. α -cubebene (13.087%), $C_{15}H_{24}$ (204), fragment ions at 204, 161, 120, 105, 91, 81, 55 with base peak 161. α -Bisabolene (9.945%), ($C_{15}H_{24}$), fragment ions at 204, 161, 135, 119, 109, 93, 79, 69, 55, with base peak 69, α -caryophyllene (7.709%), $C_{15}H_{24}$, (204), fragment ions at 204, 189, 175, 161, 147, 133, 120, 105, 93, 79, 69, 55 with base peak 93. α -Caryophyllene oxide (5.754%), $C_{15}H_{24}O$ (220), fragment ions at 220, 177, 161, 149, 135, 121, 109, 93, 79, 69, 55 with base peak 79, and copaene (3.594%) $C_{15}H_{24}$ (204), fragment ions at 204, 189, 161, 133, 119, 105, 93, 81, 77, 69, 55, with base peak 161.

Table 4Classes of compound in composition of *O. urticifolium* leaves essential oil.

Monoterpenoids ($C_{10}H_{16}$)	Sesquiterpenoids ($C_{15}H_{24}$)
α -Pinene	Copaene
3-Carene	α -Bourbonene
2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)-	α -Cubebene
2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	α -Caryophyllene
Trans-3-Carene-2-ol	α -Bergamotene
Eugenol	1,4,7,9-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z,Z,Z-
	α -Guaiane
	γ -Gurjunene
	α -Bisabolene
	δ -Cadinene
	Longipinocarveol, trans-
	(-)-Spathulenol
	Caryophyllene oxide
	Ledene oxide-(II)
	6-Isopropenyl-4,8a-dimethyl-
	1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol

3.4. Antibacterial activity of essential oil

Antibacterial activities of essential oil was determined via agar diffusion method presented in (Table 5), determined by measuring the 'inhibition zone' for essential oil of *O. urticifolium*. The activity of essential oil of *O. urticifolium* has also been compared with the broad spectrum commercially available antibiotics (tetracycline and penicillin) against one Gram-positive (*S. aureus*) and two Gram-negative (*E. coli* and *S. typhi*) bacterial strains. The result showed that the essential oil of *O. urticifolium* has high inhibitory activity on *E. coli* with activity of (10.16 ± 0.634) mm followed by *S. aureus* with activity of (5.93 ± 0.058) mm but resist on *S. typhi*. Statistically analysis of data on the antibacterial activity of essential oil on *S. aureus* and *E. coli* revealed had not

significant effect ($P > 0.05$) on the level of inhibition respect to reference antibiotic tetracycline, but *S. aureus* resist standard antibiotic penicillin. Thus the essential oil has more significant than the antibiotic penicillin and the essential oil has no effect on *S. typhi*.

Table 5Antibacterial activity of essential oil of *O. urticifolium* leaves. (mm).

	Diameter of zone of inhibition		
	<i>S. aureus</i>	<i>E. coli</i>	<i>S. typhi</i>
Essential oil	5.930 ± 0.058^a	10.160 ± 0.634^b	Resist
Tetracycline	20.200 ± 0.133^a	30.000 ± 1.876^b	22.000 ± 0.884
Penicillin	Resist	18.000 ± 0.229^b	9.000 ± 0.327

Means in the same row with the same superscripts are not significantly different ($P > 0.05$).

4. Discussion

The present work has identified various classes of phytochemical constituents in leaves extracts of *O. urticifolium*. The traditional use of this plant may be attributed to its high contents of tannins, flavonoids, steroids, terpenoids and phenols constituents. Based on this information, it could be concluded that this plant is natural sources of various bioactive secondary metabolites which helps the discovery of plant based drugs to human welfare. The physicochemical properties study of the essential oil is used as a diagnostic criterion for evaluating the purity of the oils. Acid value of the essential oil is an indirect method for determination of free fatty acid of amount in oil samples and its edibility[15]. Oil with low free fatty acids has more significant usage[16]. The acid value of the present study is in high amount of free fatty acid, not suitable for dietary purposes. The saponification value is an index of average molecular mass of fatty acid in the oil sample. The saponification value obtained for the oil samples are below the expected range of 195–205 mg KOH/g of oil for edible palm oils as specified by Pradeep *et al.* and Saleem *et al.*[17,18]. Thus the result revealed that the oil obtained from *O. urticifolium* is not recommended for dietary purpose as well as in soap industry, as these values are much lesser than the required value for soap industry of palm oil (200–205) and kernel oil (245–255)[19].

According to the literature main sesquiterpenes found in the leaf oil of *O. urticifolium* were delta-cadinene (17.2%) followed by beta-caryophyllene (14.5%) and gamma-murolene (10.5%), while the monoterpene (Z)-beta-ocimene (22.7%) and the phenyl propanoidelemicine (8.8%) were detected as other major oil components[20]. These variations indicated that the dynamics of essential composition in aromatic plants is possibly associated with the expression of different environmental effects, time of sample collecting, and methods of extraction. The previous study showed that presence of α -pinene and eugenol are well-known

components having antimicrobial activity[21,22]. Since the *O. urticifolium* essential oil was mainly constituted of α -pinene and eugenol, the antibacterial activity of the oil could be attributed mainly to these compounds.

GC-MS is a good tool for quantitative and qualitative analysis of volatile oils. Phytochemical screening of crude extract of *O. urticifolium* revealed that the presence of tannins, glycosides, saponins, flavonoids, steroids, terpenoids, flavonoids and phenols. The chemical constitution of the essential oil of *O. urticifolium* was different from region to region due to the effect of geographical and environmental conditions on the volatile compositions of the ingredients. *In vitro*, antibacterial study of the crude leaf oil of *O. urticifolium* indicated considerable activity against Gram-positive (*S. aureus*) bacteria and Gram-negative (*E. coli*) bacteria. The antibacterial activity of *O. urticifolium* leaf oil is mainly due to presence of monoterpenoids like α -pinene and eugenol. Further studies of this plant species should be carried out to investigate the isolation and characterization bioactive compounds in support of its traditional use.

Conflict of interest statement

We declare that we have no conflict of interest.

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