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Chemical composition and biological activities of the essential oil of *Laggera aurita* Linn (DC.) grown in Pakistan

[Pakistan'da Yetişen *Laggera aurita* Linn (DC.) esansiyal yağlarının kimyasal kompozisyonu ve biyolojik aktivitesi]*

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ABSTRACT

Aim: *Laggera aurita* Linn (DC.) (Asteraceae), an aromatic weed, has significant ethno medicinal use in China. This study was designed to determine xanthine oxidase, antioxidant, and antibacterial activities of the steam distilled volatile oil of *Laggera aurita* grown in Pakistan.

Materials and methods: The volatile oil of the aerial parts of *L. aurita* was obtained by hydrodistillation and analyzed by GC-MS. The biological activities investigated include xanthine oxidase inhibition and antioxidant activities using UV spectrophotometry and antibacterial activity through agar well diffusion method.

Results: GC-MS analysis of the steam distilled volatile oil of *L. aurita* showed highest concentration of fatty acids (47.5%), followed by sesquiterpenes (30.8%) and hydrocarbons (8.6%). Monoterpenes and phenolic derivatives were observed as the minor components. The volatile oil exhibited significant xanthine oxidase inhibition potential with IC_{50}=0.45 ± 0.009 mg/ml. A dose-dependent increase in the antioxidant potential was observed in DPPH$^{\bullet}$ radical scavenging and total antioxidant assays Among the four selected strains, the essential oil was found more active against *Proteus mirabilis* (MIC=0.5 mg/ml) *and Bacillus subtilis* (MIC=1.0 mg/ml).

Conclusion: Results of the present study show that the volatile oil of *Laggera aurita* has significant xanthine oxidase inhibitory potential. In addition, the oil showed high results in the antibacterial assay, comparable with the standard. Further investigation is necessary for the identification of active component.

Key Words: *Laggera aurita*, xanthine oxidase, antioxidant and antibacterial

Conflict of Interest: There is no conflict of interest among the authors who contributed to the present study.

ÖZET

Amaç: Aromatik bir ot olan *Laggera aurita* Linn (DC.) (Asteraceae), Çin'de tıpta özgül etno kullanımı bulunmaktadır. Bu çalışma, Pakistan'da yetişen *Laggera aurita* uçucu yağının, Ksantin Oksidaz üzerine antioksidant etkisini ve bu yağın antimikrobiyal aktivitelerini incelemek için yapılmıştır.

Gereç ve Yöntemler: *L. aurita* havayla ilişkin kısımlarının uçucu yağları, hidrodistilasyon yöntemi ile elde edilerek, GC_MS ile analiz edilmiştir. Ksantin oksidaz'ın inhibisyon düzeyinde biyolojik aktiviteleri ve uçucu yağın antioksidant aktivitesi, sırasıyla UV spektrofotometre ve agar difüsyon metodu ile analiz edilmiştir.

Bulgular: GC-MS ile *L. aurita*'nın uçucu yağının yüksek konsantrasyonda serbest yağ asidi miktarına (%47.5), %30.8 oranında seskiterpene ve %8.6 oranında hidrokarbonlara sahip olduğu gösterilmiştir. Monoterpenler ve fenolik türevler minor komponentler olarak gözlenmişlerdir. Uçucu yağ, IC_{50}=0.45 ± 0.009 mg/ml oranında bir inhibisyon potensiyali ile Ksantin oksidazı inhibe etmiştir. Miktara bağımlı artış ile uçucu yağın antioksidant potansiyali, radikal DPPH$^{\bullet}$ varlığında yapılmıştır. Seçilen 4 bakteri suju arasında, *Proteus mirabilis* (MIC=0.5 mg/ml) ve *Bacillus subtilis* (MIC=1.0 mg/ml)'a karşı en fazla aktif olduğu belirlenmiştir.

Sonuçlar: Çalışmanın sonucu olarak, *Laggera aurita* uçucu yağının, Ksantin Oksidaz üzerine inhibisyon potansiyali olduğu gösterilmiştir. Bununla beraber, uçucu yağın, standartla karşılaştırlarak, yüksek antimikrobiyal etkisi olduğuda gösterilmiştir. Aktif komponentin belirlenmesi için ileri bir çalışma gerekmektedir.

Anahtar Kelimeler: *Laggera aurita*, Ksantin oksidaz, antioksidant ve antimikrobiyal etkileri

Çıkar çatışması: Katkıda bulunan yazarların hiçbir çıkar çatışması bulunmamaktadır.

Introduction

Living cells may generate free radicals and reactive oxygen species (ROS) such as superoxide radicals during normal aerobic metabolism of biological systems [1]. Various diseases such as arthritis, mongolism, asthma, carcinoma, dementia, and Parkinson's disease are caused by ROS [2]. Xanthine oxidase (XO), a flavoprotein enzyme, is considered to be an important biological source of superoxide radicals, which contribute to the oxidative stress on the organism and are involved in many pathological processes such as inflammation, atherosclerosis, cancer, aging, etc. It plays a key role in the pathway for purine breakdown and conversion of xanthine into uric acid which ultimately results in a painful inflammation known as gout [3]. The free radicals and ROS are scavenged and deployed by the antioxidants. Essential oils have been widely used for the treatment of different diseases including inflammations, cancers and cardiovascular diseases, in atherosclerosis and thrombosis [4]. Recently, there is a growing interest in oxygen-containing free radicals in biological systems and their implied role as causative agents in the etiology of a variety of chronic disorders [5]. Therefore, attention is focused on the protective effects of naturally occurring antioxidants in the cells of the organisms containing them [6-7]. Antioxidants in the volatile oils are important in the stabilization of free fatty acids [8-9]. The antioxidant activity of phenols and other compounds present in the volatile oils has been widely studied by many authors [10-11].

Laggera aurita Linn (DC.) belongs to the family Asteraceae and the genus *Laggera* is comprised of approximately 20 species and mainly found in tropical Africa and Southeast Asia [12]. Only two species of *Laggera* are found in Pakistan among which *L. alata* is rare and only found in District Hazara, while *L. aurita* is abundantly found in Sind and Punjab [13]. Previous investigation on the volatile oil of *L. aurita* from India has led to the identification of seven constituents [14], while 52 constituents from volatile oil of *L. aurita* from Burkina-Faso [15]. Bactericidal properties of the volatile oil are reported by Geda in 1995 [16]. Other species of *Laggera* such as *L. pterodonta* has been employed in herbal medicine in China as anti inflammatory agent for treatment of hepatitis, arthritis, bronchitis and nephritis [17-18]. However, no significant work has been reported on chemical composition and bioactivity related studies of the species found in Pakistan. Therefore, the present study was carried out, to determine xanthine oxidase, antioxidant and antibacterial potential of the volatile oil of *L. aurita* grown in Pakistan.

Materials and Methods

Plant material

Laggera aurita Schulz was collected (January, 2009) from Shahdara Lahore, Pakistan. The plant was identified at the Department of Botany, GC University Lahore, where a voucher specimen was submitted (GC-Herb-Bot-240).

Isolation of essential oil

The aerial parts of *L. aurita* (500g) were chopped and subjected to steam distillation using Dean Stark apparatus. The volatile oil was collected, dried with anhydrous sodium sulfate and kept at 4◦C (% yield = o.43 %, w/w).

GC-MS Analysis

GC-MS analysis of the volatile oil was carried out with a Shimadzu GCMS-QP 2010 system (30 m, 0.25 mm, 0.25 µm film thickness). GC oven temperature was programmed at 50°C (initial temperature maintained 2 min) to reach 160°C at a rate of 5°C/min, kept constant 5 min, then programmed to reach 270°C at a rate of 10°C/min, where it is kept constant for 10 min. The carrier gas (Helium 99.95%) was set at a constant flow of 1 ml/min. A 2-µl sample volume was injected manually with split ratio adjusted at 40:1. Mass spectra were recorded at 70 eV. The mass range was from *m/z* 35 to 450.

Identification of the essential oil components

The compounds were identified by comparison of their mass spectrum to those of NIST MS library version 2.0.

Xanthine oxidase inhibitory (XOI) activity

The assay was performed spectrophotometrically under aerobic conditions with slight modification [19]. The assay mixture consisted of different volume (0.013 ml to 0.05 ml) of test oil solution in (4 mg/ml in DMSO), 0.330 ml of xanthine solution (0.15 mM) and 50 mM phosphate buffer (pH 7.5) to make the volume of assay mixture up to 2 ml. The reaction was initiated by adding 0.035 ml XO solution and then absorbance was recorded at 295 nm after adding 0.1 ml of 1N HCl. XOI activity was expressed as % inhibition of XO in the above assay system, calculated as:

$$\% \text{ inhibition of XO} = (Ab_{enzyme} - Ab_{blank}) - (Ab_{enzyme+e.\ oil} - Ab_{e.\ oil}) / (Ab_{enzyme} - Ab_{blank}) \times 100$$

Where Ab_{enzyme} is the absorbance of enzyme without test volatile oil, Ab_{blank} is the control without test essential oil and enzyme, $Ab_{e.\ oil}$ is the absorbance of the test volatile oil while $Ab_{enzyme+e.\ oil}$ is the absorbance of the volatile oil with XO. Allopurinol was used as a positive control. IC_{50} values were calculated from the mean value of data.

Antioxidant potential

DPPH radical scavenging assay

Antioxidant activity of the volatile oil was measured in terms of radical scavenging ability using DPPH• [20]. Methanolic solution (1ml) at various concentrations

(0.05-4mg/ml) was added to 2ml (0.1 mg/ml) of DPPH solution in methanol and incubated in dark at room temperature for 30 minutes. The decrease in absorbance at 517 nm was determined and the results were evaluated as % scavenging of radical (% scavenging of $DPPH^{\bullet}$ = Absorbance of blank – Absorbance of sample/ Absorbance of blank × 100). IC_{50} value (concentration of sample where absorbance of DPPH decreases 50 % with respect to absorbance of blank) of the volatile oil was also calculated.

Total antioxidant activity

Total antioxidant activity of the volatile oil was evaluated by the formation of phosphomolybdenum complex [21]. 0.1 ml solution of the volatile oil in methanol (0.05-4mg/ml) was combined with 1.9 ml of reagent solution (0.6 M H_2SO_4, 28 mM sodium phosphate and 4mM ammonium molybdate). The blank contained 2 ml of reagent solution. The vials were capped and incubated at 95 C° and absorbance was measured at 695 nm after an hour.

Antibacterial activity

Different concentrations of the volatile oil were tested against four microorganisms including *Bacillus subtilis* PTCCBS, *Escherichia coli* ATCC8789, *Nocardia asteroides* PTCCNA and *Proteus mirabilis* ATCC0321P, which were obtained from the PCSIR LABS Complex, Lahore. Antibacterial activity of *L. aurita* volatile oil was evaluated using an agar well diffusion method [22]. Inoculum suspensions of different microorganisms were prepared in sterilized nutrient broth solution, added to 2.5 % sterilized solution of nutrient agar and poured in sterilized petri plates. 5 mm-diameter wells were cut from the agar using a sterile cork-borer and 100 µl different concentrations of the volatile oil in DMSO were placed in the wells. The plates were then incubated for 24 hours at 37°C. Antibacterial activity was evaluated by measuring zones of inhibition in mm and compared with the standard.

Statistical analysis

Statistics was applied by Microsoft Excel 2003. All the experiments were run as triplicate and presented here as average ± confidence level (a = 0.05 and n = 3).

Results

GC-MS analysis of the crude volatile oil led to the identification of 19 constituents representing 87.3% of the total composition of the oil (Table 2). There were five fatty acids (47.5%), six sesquiterpene (30.8%), and four hydrocarbons (8.6%). Monoterpenes and phenolic derivatives were found as minor components. Linalool formate, β-farnesene, α-calacorene, tridecanoic acid, tetradecanoic acid, 4,8,12-tetradecatrien-1-ol, 5,9,13-trimethyl-, hexadecanoic acid, 9,12-octadecadienoic acid, octadecanoic acid, heptadecane, 1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester, eicosane and pentadecane have been reported for the first time, while six constituents β-caryophyllene, dimethoxy-p-cymene, α-caryophyllene, 10s,11s-himachala-3(12),4-diene, T-cadinol and α-cadinol were already reported from the volatile oil of *L. aurita* (35.1% of the total oil composition).

Concentration dependant assay was carried out to determine IC_{50} of essential oil ((IC_{50}=0.045 ± 0.009 mg/ml). The volatile oil of *L. aurita* showed comparable XO inhibition potential with allopurinol ((IC_{50}=0.009 ± 0.003 mg/ml) which was used as standard (Table 2).

Concentration dependant assay was carried out to evaluate radical scavenging potential of the volatile oil in the range of 0.05 to 4 mg/ml. The l oil showed moderate radical scavenging ability with IC_{50} =2.28 ± 0.07 mg (Table 2). Total antioxidant activity of the oil is measured in terms of its ability to reduce Mo (VI) phosphate complex to Mo(V) phosphate complex and the results are shown in Table 2 (Fig 1). The volatile oil of *L. aurita* showed total antioxidant activity comparable to that of BHT. Antibacterial activity of the volatile oil was tested against *B. subtilis, E. coli, N. asteroides* and *P. mirabilis* by agar well diffusion method. Different concentrations of volatile oil (0.5-100 mg/ml) in DMSO were tested against each microorganism. The oil was most active against *P. mirabilis* and least active against *N. asteroides* (Table 3, Fig 2).

Discussion

The results of GC-MS analysis as shown in Table 1 suggested that the volatile oil of *L. aurita* grown in Pakistan has significantly different chemical composition than the volatilel oil of the same species from other countries already reported.The essential oil of *L. tomentosa* differed from the essential oil of the other species in the genus. The main constituents of the essential oil were chrysanthenone, isochrysanthenone, filifolone and (Z) isogeranic acid, which were not reported from other *Laggera* species [23]. The composition of *L. pterodonta* essential oil from Cameroon was found to contain γ-eudesmol, α-eudesmol, 2,5-dimethoxy-p-cymene and juniper camphor as main components [24]. *L. alata* essential oil from Madagascar contained 7-epi-γ-eudesmol, 7-epi-β-eudesmol, 7-epi-α-eudesmol and juniper camphor as its major constituents [25]. The essential oil of *L. alata* from Cameroon, contained 2,5-dimethoxy-p-cymene and sabinene as major components, while essential oil samples from other regions of Nigeria were reported to contain thymoquinol dimethyl ether, α-eudesmol and juniper camphor as main constituents. *L. gracilis* essential oil from Cameroon contained dimethoxy-p-cymene, γ-eudesmol, β-caryophyllene, germacrene-D and juniper camphor as major constituents. The essential oil of *L. oloptera* from Cameroon was rich in sabinene, limonene, β-caryophyllene, germacrene-D, γ-eudesmol and dimethoxy-p-cymene [26].

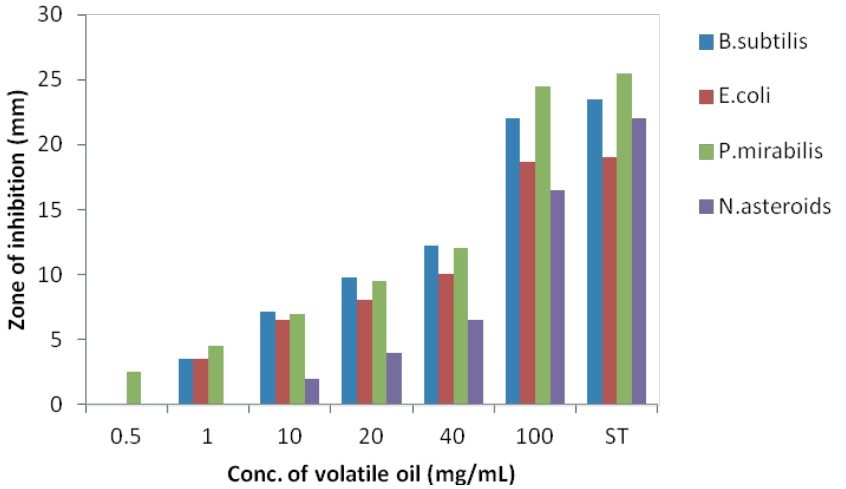

Figure 1. Antibacterial activity of the volatile oil of *Laggera aurita.*

Table 1. Chemical composition of the volatile oil of *Laggera aurita*

Compound name	%	[a]Identification method
linalool formate	0.1	MS
β-caryophyllene	0.3	MS
dimethoxy-p-cymene	4.4	MS
α-caryophyllene	0.5	MS
β-farnesene	0.1	MS
10s,11s-himachala-3(12),4-diene	3.6	MS
α-calacorene	0.2	MS
T-cadinol	11.7	MS
α-cadinol	14.6	MS
tridecanoic acid	7.1	MS
tetradecanoic acid	0.1	MS
4,8,12-tetradecatrien-1-ol, 5,9,13-trimethyl-	0.3	MS
hexadecanoic acid	21.2	MS
9,12-octadecadienoic acid	14.3	MS
octadecanoic acid	3.6	MS
heptadecane	0.2	MS
1,2-benzenedicarboxylic acid, mono (2-ethylhexyl) ester	1.2	MS
eicosane	0.2	MS
pentadecane	3.6	MS

[a]Mass Spectral similarity with NIST MS library database Version 2.0

Table 2. XO inhibitory potential, DPPH radical scavenging ability and total antioxidant activity of the volatile oil of *Laggera aurita*.

Conc. of volatile oil (mg/ml)	Xanthine oxidase inhibition (%)	DPPH· Scavenging (%)	Total antioxidant activity (Absorbance at 695 nm)
4	-	81.40 ± 1.98	1.72 ± 0.16
2	-	45.01 ± 2.69	1.09 ± 0.23
1	-	26.06 ± 4.20	0.80 ± 0.05
0.5	-	17.56 ± 1.82	0.57 ± 0.07
0.1	79.05 ± 7.39	13.46 ± 5.00	0.40 ± 0.02
0.05	59.93 ± 8.39	7.59 ± 3.28	0.26 ± 0.02
0.025	33.86 ± 7.24	-	-
BHT	-	91.17 ± 1.75	0.69 ± 0.08
Allopurinol	96.79 ± 2.47	-	-

Data is average (average ± Confidence level at α=0.05) of three parallel readings.
BHT =0.5mg/ml; Allopurinol =0.1 mg/ml

Table 3. Antibacterial activity of the volatile oil of *Laggera aurita*.

Volatile oil conc. (mg/ml)	Zone of Inhibition (mm)			
	B. subtilis	***E. coli***	***P. mirabilis***	***N. asteroids***
100	22 ± 1.5	18.7 ± 2.9	24.5 ± 1.7	16.5 ± 1.5
40	12.2 ± 0.9	10.0 ± 1.2	12.0 ± 0.5	6.5 ± 0.6
20	9.8 ± 0.3	8.0 ± 0.5	9.5 ± 0.3	4 ± 0.2
10	7.1 ± 1.2	6.5 ± 0.5	7.0 ± 0.2	2.0 ± 0.1
1	3.5 ± 0.6	3.5 ± 0.1	4.5 ± 0.2	-
0.5	-	-	2.5 ± 0.0	-
Streptomycin (1mg/ml)	23.5 ± 1.5	19.0 ± 2.1	25.5 ± 2.5	22.0 ± 3.0

Data is average (average ± Confidence level at α=0.05) of three parallel readings.
-; no activity.

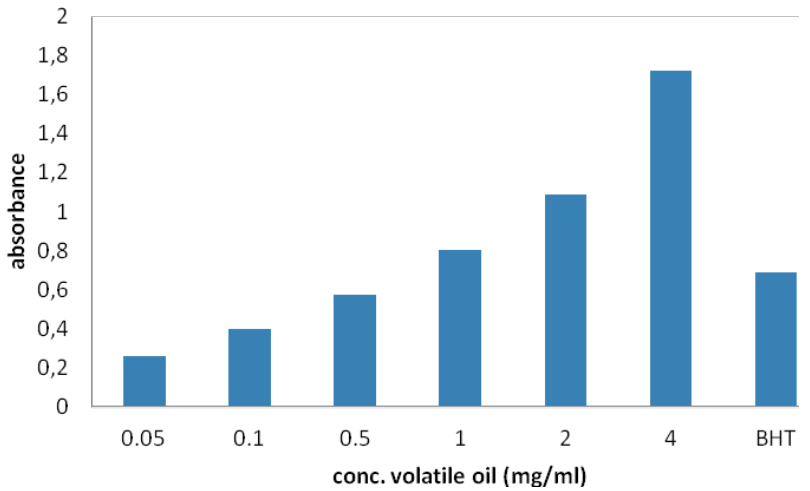

Figure 2. Total antioxidant activity of the volatile oil of *Laggera aurita*.

The volatile oil of *L. aurita* from India has been reported to contain laggerol, 2,3-dimethoxy-p-cymene, δ-cadinene, α-cadinol and *m*-menth-6-en-8-ol as the major constituents [14]. The volatile oil from Burkina-Faso was reported to contain dimethoxy-*p*-cymene, α-humulene, β-caryophyllene and longipinene derivative as the major constituents [15]. The results of the present study showed the presence of hexadecanoic acid, 9, 12-octadecadienoic acid, α-cadinol, T-cadinol and dimethoxy-*p*-cymene as the major constituents in the volatile oil of *L. aurita* (Table 1). XO catalyses the oxidation of xanthine into uric acid. Overproduction of uric acid causes gout which is one of the most common metabolic disorders affecting human. This study was conducted to investigate XO inhibitory potential of *L. aurita* volatile oil. As shown in Table 2, the volatile oil exhibited significant XO inhibition (IC_{50}=0.045 ± 0.009 mg/ml), comparable with allopurinol. Moderate results in the DPPH radical scavenging activity can be attributed to the absence of phenolics in the volatile oil. Moreover, it can be suggested that the observed activity may be due to the synergistic action of all the compounds present in the volatile oil [5, 27-28]. Conversely, total antioxidant activity is sensitive to a wide range of compounds, including phenolics, tocopherols, terpenes, and ascorbic acid [29], which is evident in the result (Table 2). Results of the antimicrobial activity are shown in Table 3. It was determined that the oils had varying degrees of growth inhibition against the selected bacterial strains The Gram-positive strains showed more susceptibility to the tested oil than the Gram-negative ones. The volatile oil of *L. aurita* demonstrated the highest activity against *P. mirabilis* with lowest MIC value (0.5 mg/mL) while MIC values were higher than 0.5 mg/mL against all other organisms (Table 3) . It has been reported that oxygenated monoterpenes are responsible for the antimicrobial activity of several volatile oils [30-35]. Moreover, sesquiterpene particularly oxygenated sesquiterpenes possess a wide range of biological activities [36]. Therefore, in the present investigation,

biological activities of the volatile oil of *L. aurita* can be attributed to the high percentage of α- cadinol and T-cadinol [37]. Possible synergistic effect of some other compounds in the oils such as hexadecanoic acid and 9,12-octadecadienoic acid should also be taken in consideration [38-39].

Conclusion

Our results suggested that the composition of the volatile oil of a particular plant species may vary considerably due to difference of their origin and environmental conditions. Furthermore, volatile oil of *L. aurita* can be useful in the treatment of gout and diseases caused by bacterial infection, and over production of free radicals.

Conflict of Interest: There is no conflict of interest among the authors who contributed to the present study.

References

[1] Harman D. (1998) Aging: phenomena and theories. Ann NY Acad Sci . 854**:** 1-7.

[2] Perry G, Raina AK, Nunomura A, Wataya T, Sayre LM, Smith MA. (2000) How important is oxidative damage lessons from Alzheimer's disease. Free Radical Bio Med. 28(5): 831–834.

[3] Ahmad N, Shahwar D, Khan MA, Mughal MS. (2010) Antioxidant potential and xanthine oxidase inhibitory activity of *Croton sparsiflorus*. Asian J Chem. 22 (1): 555-561.

[4] Edris AE. (2007) Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: A review. Phytother Res. 21: 308–323.

[5] Candan F, Unlu M, Tepe B, Daferera D, Polissiou M, Sokmen A, Akpulat HA. (2003) Antioxidant and antimicrobial activity of the essential oil and methanol extracts of *Achillea millefolium* subsp. millefolium Afan. (Asteraceae). J Ethnopharmacol. 87: 215-220.

[6] Larson RA. (1988) The antioxidants of higher plants. Phytochem. 27: 969-978.

[7] Halliwell B. (1997) Antioxidants and Human Disease: A General Introduction . Nut Rev. 55: S44.

[8] Six P. (1994) Current research in natural food antioxidants. International News on Fats, Oils & Related Materials.5: 679-68.

[9] Baldioli M, Servili M, Perretti G, Montedoro GF. (1996) Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. J Am Oil Chem Soc. 73: 1589-1593.

[10] Litridou M, Linssen J, Schols H, Bergmans M, Posthumus M, Tsimidou M, Boskou D. (1997) Phenolic compounds in virgin olive oils: fractionation by solid phase extraction and antioxidant activity assessment. J Sci Food Agri. 74: 169-174.

[11] Visioli F, Bellomo G, Galli C.(1998) Free radical-scavenging properties of olive oil polyphenols. Biochem Biophys Res Comm. 247: 60-64.

[12] Zheng Q, Xu Z, Sun X, Yao W, Sun H, Cheng CHK, Zhao Y. (2003) Eudesmane and megastigmane glucosides from *Laggera alata*. Phytochem.63: 835–839.

[13] Akbar AM, Mir AK. (2009) Pollen Morphology of rare taxa *Laggera alata a*nd its related species *Pluchea lanceolata* of tribe Pluchеeae (ASTERACEAE). Pak J Bot. 41(4): 1539-1544.

[14] Zutshi SK, Bamboria BK, Bokadia MM.(1975) Chemical investigation of the essential oil of *Laggera*. Curr Sci. 44: 571-572.

[15] Mevy JP, Bousquet-Melou A, Greff S, Millogo J,Fernandez C. (2006) Chemical composition of the volatile oil of *Laggera aurita* Schulz from Burkina-Faso. Biochem System Ecol. 34: 815-818.

[16] Geda AK. (1995) Antibacterial activity of essential oils and their combinations. Fett Wiss Fat Sci Technol. 97 (12): 458-461.

[17] Wu Y, Wang F, Zheng Q, Lu L, Yao H, Zhou C, Wu X and Zhao Y. (2006) Hepatoprotective effect of total flavonoids from *Laggera alata* against carbon tetrachloride-induced injury in primary cultured neonatal rat hepatocytes and in rats with hepatic damage. J Biomed Sci. 13: 569-578.

[18] Wu Y, Yang L, Wang F, Wu X, Zhou C, Shi S, Moban J, Zhao Y (2007) Hepatoprotective and antioxidative effects of total phenolics from *Laggera pterodonta* on chemical-induced injury in primary cultured neonatal rat hepatocytes. Food Chem Toxicol. 45(8): 1349-1355.

[19] Owen PL, Johns T. (1999) Xanthine oxidase inhibitory activity of northeastern North American plant remedies used for gout. J Ethnopharm. 64:149-160.

[20] Shahwar D , Raza MA, Saeed A S, Riasat M, Chattha FI, Javaid M, Ullah S, Ullah S. (2012) Antioxidant potential of the extracts of *Putranjiva roxburghii*, *Conyza bonariensis*, *Woodfordia fruiticosa* and *Senecio chrysanthemoids.* Afr J Biotech. 11(18): 4288-4295.

[21] Shahwar D , Ullah S, Raza MA, Sana U,Yasmeen A, Ghafoor S , Ahmad N. (2011) Acetylcholine esterase and antioxidant potential of some members of Asteraceae and Euphorbiaceae. J Med Plants Res. 5(32): 7011-7016.

[22] Ayaz FA, Hayirliglu-A S, Aplay-K S, Gruz J, Valentova K, Ulrichova J, Strnad M. (2008) Phenolic acid contents of kale (*Brassica oleraceae* L var acephala DC) extracts and their antioxidant and antibacterial activities. Food Chem. 107: 19-25.

[23] Asfaw N, Storesund HJ, Aasen AJ, (2003) Constituents of the Essential Oil of *Laggera tomentosa* Sch. Bip. ex Oliv. et Hiern Endemic to Ethiopia**.** J Essent Oil Res. 15(2): 102-105.

[24] Ngassoum MB, Jirovetz L, Buchbauer G, Fleischhacker W. (2000) Investigation of the Essential Oil and Headspace of*Laggera pterodonta* (DC.) Sch. Bip. ex Oliv., a Medicinal Plant from Cameroon. J. Essent. Oil Res. 12(3): 345-349.

[25] Ekundayo O, Oguntimein B, Laakso I, Hiltunen R, (1989) Composition of the Essential Oil of *Laggera alata*. Planta Med. 55 (6): 573-574.

[26] Kuiate JR, Bessiere JM, Zollo PHA. (2002) Composition of the essential oils from three *Laggera* Spp. from Cameroon, Flavour Frag. J. 17: 105-108.

[27] Vardar-Unlu G, Candan F, Sokmen A, Daferera D, Polissiou M, Sokmen M, Donmez E, Tepe B.(2003) Antimicrobial and antioxidant activity of the essential oil and methanol extracts of *Thymus pectinatus* Fish. et Mey. Var. pectinatus (Lamidceae). J Agric Food Chem. 51: 63-67.

[28] El-Massry KF, El-Ghorab AH, Farouk A. (2002) Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L. Food Chem. 79: 331.

[29] Prieto P, Pineda M, Aguilar M. (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. Anal Biochem. 269: 337-336.

[30] Carson CF, Riley TV. (1995) Antimicrobial activity of the major components of the essential oil of *Melaleuca alternifolia*. J Appl Bacteriol. 78: 264–269.

[31] Pattnaik S, Subramanyam VR, Bapaji M, Kole CR. (1997) Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios**.** 89: 39–46.

[32] Zarrini G, Delgosha ZB, Moghaddam KM, Shahverdi AR. (2010) Post-antibacterial effect of thymol. Pharm Biol. 48: 633–636.

[33] Rúa J, Fernández-Alvarez L, Gutiérrez-Larraínzar M, del Valle P, de Arriaga D, García-Armesto MR.(2010) Screening of phenolic antioxidants for their inhibitory activity against food-borne *Staphylococcus aureus* strains. Foodborne Pathog Dis. 7: 695–705.

[34] Xu J, Zhou F, Ji BP, Pei RS, Xu N.(2008) The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. Lett Appl Microbiol. 47: 174–179.

[35] Kokoska L, Havlik J, Valterova I, Sovova H, Sajfrtova M, Jankovska I. (2008) Comparison of chemical composition and antibacterial activity of *Nigella sativa* seed essential oils obtained by different extraction methods. J Food Prot. 71(12): 2475–2480.

[36] Fraga BM. (2003) Natural sesquiterpenoids. Nat Prod Rep. 20: 392–413.

[37] Cheng SS, Wu CL, Chang HT, Kao YT, Chang ST.(2004) Antiemetic and antifungal activities of essential oil of *Calocedrus formosana* leaf and its composition. J Chem Ecol. 30:1957-1967.

[38] Dubey NK, Kishore N. (1987) Fungitoxicity of some higher plants and synergistic activity of their oils. Trop Sci. 27: 23-27.

[39] Delamare APL, Moschen-Pistorello IT, Artico L, Ahi-Serafini L, Echeverrigaray S. (2007) Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in South Brazil. Food Chem. 100: 603-608.