Chemical Composition and Bioactivity of Essential Oil of *Ageratina adenophora* from Bhaktapur District of Nepal

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Abstract

The essential oil of *Ageratina adenophora* locally call Banmara was analyzed by GC–MS. A total of 45 volatile compounds was identified and torreyol (16.8%) was the major component in oil. The sesquiterpene fraction was higher than the monoterpene one. The essential oil showed significant antibacterial activity against both gram positive (*Klebsiella pneumoniae* and *Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Proteus vulgaris*) bacteria.

Key words: *Ageratina adenophora*, *Banmara*, essential oil, physiochemical parameters, *Eupatorium adenophorum*, antibacterial activity and Zone of Inhibition

Introduction

Eupatorium adenophorum (syn. *Ageratina adenophora*, common name: Crofton weed; Sticky snakeroot), a native of Mexico has naturalized in many countries[1]. *A. adenophora* belongs to the Asteraceae family. It is a perennial herb, nearly 1 meter hight, and erect, found in open and deforested areas of the central and eastern Nepal at altitude of 500-2000 m. It is a problem weed in forest plantations as it infests disturbed areas and prevents self-seeding of cultivated trees, and hence it is known as banmara in Nepal (killer of the forests)[2,3]. It grows as weed on vast expanses in many parts of the world and are an attractive source of bioactive natural products[4,5,6]. Various species of *Eupatorium* have been used in the traditional system of medicine in different parts of the world. The leaf juice of *A. adenophora* is used to stop bleeding of cut and wounds, forming clots[7]. Root juice is prescribed to treat fever. Pure juice of the leaf is poured in the eye to treat insomnia. A decoction of the plant has been recommended to treat jaundice and ulcers[4].

Extensive work on the chemical constituents as well as on the composition of the essential oils of *Argentina* is reported in literatures. *A. adenophora* has been reported for its allelopathic effect[8]. A hepatotoxic compound (9-oxo-10, 11-dehydroagerophonrone) was identified from the crude extract of *A. adenophora*, this component promotes a chronic equine respiratory disease known as "Numinbah horse"and liver lesions[9].They also found b-farnesene, germacrene D, bisabolene, caryophyllene and four more cadinene derivatives were also reported. Other compounds identified from the stems and leaves of this species were found to be 9-oxo-ageraphorone, 9-*β*-hydroxy-ageraphorone, epifriefdelinol, stigmasterol, octacosanoic acid, b-daucos-terol, *o*-hydroxycinnamic acid, ferulic acid, cafeicacid and 2-isopropenyl-5-acetyl-6-hydroxybenzo-furan acetate.There are some previous reports on the antimicrobial activity of the oils of some species of *Eupatorium*[10,11,12]. However, the natural products profile and consequently the bioactivity is known to vary with the climate and geographic location of the plants[13]. The essential oils of *Ageratina adenophora* (syn: *Eupatorium. adenophorum* (Spreng.) King and H.E. Robins) of Nepal has not been investigated so far.

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Thus, the paper presents the chemical composition, physicochemical properties and antibacterial activity of the essential oil extract from aerial part of *A. adenophorum* sample collected from Bhaktapur District, Nepal

Materials and Methods

Plant Material

About 2 kg plant material (aerial part) was collected from the Nagarkot VDC of Bhaktapur District, Nepal in February 2011. The plant was identified as *Ageratina adenophora* by using different taxonomic literatures[14,15].

Extraction of essential oil

The powdered material of aerial part of plant (100 gm) together with water (5-10 times) was taken in volumetric flask and was hyro-distilled for 6 hour using a Clevenger apparatus[16]. This process was repeated several times. The separated oil was dried over anhydrous sodium sulphate (Sigma-Aldrich,USA). Samples were stored in a refrigerator (4 ºC) until used.

Analytical condition for GC/MS

Analytical GC was recorded on gas chromatograph with a flame ionization detector using a capillary 30 m DB-5 column (J and W Scientific, USA) with 0.25 mm i.d. and 0.1 mm film thickness. The temperature program was 50^{o}C for 2 minute and increased at 10^{o}C/minute up to 300^{o}C for 3 minute. The carrier gas was Helium at a flow rate 1 ml/min. MS was operated in the electron impact mode with ionization energy of 70eV on a JEOL AX505 mass spectrometer connected to HP-9000 computer system. Most constituents were identified by comparison of their GC retention indices with those of authentic standards available in the author's laboratory or with GC data previously published [17,18,19]. Identification was confirmed when possible by comparison of their mass spectra with those stored in the MS database (NIST,Wiley libraries) and with mass spectra literature data[17,18,19,]. Relative amounts of detected compounds were calculated based on GC peak areas.

Determination of physical parameters

Physical parameters were determined according to the method of Guenther [20].

Specific gravity

An ignition tube previously cleaned and dried was weighed and its weight was determined to be W. The tube was filled with the oil and was weighed as 'W_1' . The same procedure was performed using the same tube containing water and its weight was noted as 'W_2'. Then specific gravity was calculated using the formula,

$$d_t = \frac{w_1 - w}{w_2 - w}$$

Refractive index

The refractive index of the oil was measured by using Abbe's refractometer.

Optical rotation

Different concentration of oil solutions (1.0%, 0.5%, 0.25%) were prepared in methanol and the optical rotation was measured for the solutions of different concentrations. Then the specific rotation was calculated using the formula.

$$[\alpha]^{t}_{D} = \frac{\alpha}{l \times c}$$

Where, 'α' is the angle of rotation of the plane of plane polarized Light, 'l' is the length of polarimeter tube (mm), 'c' is the concentration of oil solution and't' is the temperature at the time of measurement.

Determination of chemical parameter

Saponification value

Saponification value was determined by standard procedure. *Ageratina* essential oil (0.5 gm) was accurately weighed in a 250 mL conical flask and dissolved in 10 mL of ethanol and 2.5N potassium hydroxide (KOH) solution was added. This procedure was performed in duplicate and blank experiment was also performed omitting the oil. The mixture was refluxed for two hours then cooled. The unreacted KOH was titrated with standard N/2 oxalic acid by adding 2-3 drops of phenolphthalein indicator. Then, the saponification value was determined using the following equation,

$$\text{Saponification value} = \frac{56 \text{ X } (V_1\text{-}V_2) \text{ X}}{2 \text{ X } 1000 \text{ X}}$$

Where, 'W' is the weight of oil, 'V_1' is the volume of N/2 oxalic acid for blank and 'V_2'is the volume of N/2 oxalic acid for sample

Acid value

Acid value was determined according to the method of Guenther [20]. Oil (0.5 gm) was accurately weighted into a 250 mL conical flask and dissolved in 10 mL of neutral 95% ethanol and 2-3 drops of phenolphthalein indicator was added. The free acid was then titrated with standard 0.1 N aqueous sodium hydroxide solution by adding the alkali drop-wise at a uniform rate of about 30 drops per minute. The content of the flask was continuously agitated. The first appearance of the red coloration that did not fade within 10 seconds was considered the end point. Then, the acid value (A.V) was calculated using the following equation,

$$\text{A.V.} = \frac{5.61 \text{ (Number of mL of 0.1N NaOH)}}{\text{Weight of sample in gram}}$$

Iodine number

Iodine number was determined according to the method of Guenther [20]. Oil (0.25 gm) was dissolved in 10 mL of chloroform. Then 25 mL of iodobromide solution was added and allowed to stand for 30 minutes in dark. Again 30 mL of 1N potassium iodide and 100 mL of distilled water were added and the liberated iodine was titrated with N/10 solution of sodium thiosulphate with constant shaking. When iodine color became quite pale, 1 mL of 1% starch solution was added and the titration was continued until the blue color was discharged. A blank test was also carried out parallel under identical condition. The iodine number was determined using the formula,

$$\text{Iodine number} = \frac{1.269 \text{ } (V_1\text{-}V_2)}{W}$$

Where, 'W' is the weight of sample, 'V_1' is the number of mL of sodium thiosulphate consumed by the blank, 'V_2' is the number of mL of sodium thiosulphate consumed by the test sample.

Iodobromide solution was prepared by dissolving iodine (13.2 gm) in 1000 mL glacial acetic acid by gentle heating. The solution was cooled to 25°C and the iodine content in 20 mL was determined by titration with N/10 Sodium thiosulphate. To the remaining of the solution a quantity of bromine molecularly equivalent to that of the iodine present was added.

Antibacterial Screening

Inhibition of bacterial growth was tested by using the paper disc diffusion method with slight modification[21].

Micro organism

The micro organisms used in this study were identified strains obtained from Central Department of Microbiology, T.U. Among bacteria taken in this study, two were gram positive and two were gram negative as given below.

Gram positive bacteria: *Klebsiella pneumoniae* and *Staphylococcus aureus*

Gram negative bacteria: *Escherichia coli* and *Proteus vulgaris*

Antimicrobial activity

The anti microbial activity of the essential oil was carried by disc diffusion method[21]. A suspension of tested micro organisms was spread on Muller-Histon Agar (MHA) medium. The sterile filter paper discs (6 mm in diameter) were individually impregnated with different concentration of oil prepared in ethanol and then placed into the agar plates which had previously been inoculated with the tested micro organisms. The plates were subsequently incubated overnight at 37°C. After incubation the growth inhibition rings were quantified by measuring the diameter of the zone of inhibition in mm. For control petroleum ether discs were used. All tests were performed in triplicate.

Results and Discussion

The essential oil obtained by hydrodistillation of the aerial part of *A. adenophora* was slightly viscous, whitish in color and with characteristic odor. The yield of the oil was 4.5% on the fresh weight basis. Gas chromatophraphy analysis of the essential oil showed the presence 45 components and the major compound was found to be torreyol (16.8%). Other representative components of the oil were identified as 2-pentanone (7.71%), germacrene (7.49%), bornyl acetate (7.51%), 1-α -bisabolene (6.82%), δ-cadinene (6.4%), α-bisabolol (5.1%). The complete list of identified compounds of the oil, their retention time and percentage composition are given in Table 1. The earlier investigation of the essential oil of *E. adenophorum* leaves from India shows 1-napthalenol (17.50%), α-bisabolol (9.53%), bornyl acetate (8.98%), β-bisabolene (6.16%), germacrene-D (5.74%) , α-phellandrene (3.85%) and a di-*epi*-α-cedrene (2.98%)[12]. Weyerstahl and co-workers from India (1997) reported the composition of the essential oil of flowers of *E. adenophorum*[22]. The major constituents were α-phellandrene (15.3%), camphene (12.2%), bornyl acetate (10.6%), *p*-cymene (8.5%), γ-curcumene (4.5%) and 2-carene. Pala-Paul and coworkers (2010) reported the analysis of the essential oil of the aerial parts of *A. adenophora*[24]. The major constituents were *p*-cymene (11.6%), α-phellandrene (5.7%), γ-curcumene (5.0%), δ-2-carene (5.0%), camphene (4.8%), and endo-bornyl acetate (4.4%)[23]. The composition of the essential oil from the aerial part of *A. adenophora* obtained here is supported by similar previous reports on this species from different countries. They reported the oils of *Ageratina (= Eupatorium)* are richer in sesquiterpenes than in monoterpene compounds. However, there is remarkable difference in the nature and relative

content of different constituents in the previous reports by Papa-paul et al 2010 and the data on Table 1. The sample used in this study and those in the previous studies might be representing different chemotypes.

Table 1. *Percentage Composition of Essential oil from the aerial parts of A. adenophora*

SN	Compounds	Retention Time	Area %
1	2-Pentanone, 4-hydroxy-4-methyl- (CAS) Diacetone alcohol	5.733	7.71
2	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene- (CAS) 3,3-Dimethyl-2-methylenenorbornane	8.058	0.4
3	*p*-Mentha-1,4(8)-diene	9.183	0.73
4	1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)- (CAS) p-Mentha-1,5-diene	9.267	1.05
5	Benzene, methyl(1-methylethyl)- (CAS) Cymol	9.717	1.96
6	*β*-Linalool	11.325	0.22
7	endo-2-Hydroxy-1,7,7-trimethylnorbornane	12.892	0.53
8	*p*-Mentha-1,5-dien-8-ol	13.417	0.24
9	Bornyl acetic ether	15.325	7.51
10	Camphene	16.842	0.42
11	Bicyclo[2.2.1]heptane, 2,2-dimethyl-3-methylene- (CAS) 3,3-Dimethyl-2-methylenenorbornane	16.975	0.24
12	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8-methylene-, [1R-(1R*,4E,9S*)]	18	1.91
13	2-Norpinene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	18.167	2.07
14	beta.-Sesquiphellandrene	18.283	0.72
15	(6Z)-7,11-Dimethyl-3-methylene-1,6,10-dodecatrien	18.433	3.39

16	1H-Benzocycloheptene, 2,4a,5,6,7,8-hexahydro-3,5,5,9-tetramethyl-, (R)-	18.933	1.64
17	δ-Cadinene	19.017	6.4
18	Germacrene D	19.1	7.59
19	trans-Caryophyllene	19.158	1.49
20	1,2,3,4,5-Pentamethylcyclopentadiene	19.383	1.42
21	β-Bisabolene	19.442	6.81
22	Tricyclo[7.2.0.0(3,8)]undec-4-ene, 4,8,11,11-tetramethyl	19.6	0.56
23	β-Sesquiphellandrene	19.733	2.18
24	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)- (CAS) (+)-.delta.-Cadinene	19.783	0.43
25	5-Isopropyl-2-methylbicyclo[3.1.0]hex-3-en	19.833	0.65
26	(3E)-4,4-Dimethyl-3-(3-methyl-3-butenylidene)-2-m	19.908	1.11
27	Cyclohexene, 4-(1,5-dimethyl-1,4-hexadienyl)-1-methyl-	20.017	0.7
28	Androstan-17-one, 3-ethyl-3-hydroxy-, (5.alpha.)	20.242	1.05
29	(6E)-3,7,11-Trimethyl-1,6,10-dodecatrien-3-ol	20.333	0.33
30	Andrographolide	20,508	0.65
31	(-)-Spathulenol	20.817	0.98
32	Caryophyllene oxide	20.942	0.94
33	α-Cedrol	21.258	0.47
34	Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl	21.517	20.79
35	Guaiol	21.583	0.83
36	Torreyol	21.725	16.8
37	Isoledene	21.808	0.75

38	Azulene, 1,2,3,4,5,6,7,8-octahydro-1,4-dimethyl-7-(1-methylethylidene)-, (1S-cis)- (CAS) Guaiene	22.067	0.98
39	α-Bisabolol	22.45	5.1
40	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0(2,4)]octane	22.633	1.11
41	9H-Cycloisolongifolene, 8-oxo	22.75	4.8
42	Cyclodecacyclotetradecene, 14,15-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro	23.15	0.51
43	Bicyclo[4.4.0]dec-5-ene, 1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)	24.35	1.15
44	1,4,4,7a-Tetramethyl-2,4,5,6,7,7a-hexahydro-1H-indene-1,7-diol	26.367	0.36
45	Di-epi-α-cedrene	27.192	0.28

The physicochemical property of the oil was evaluated using the standard procedure and the result is presented in Table 2. The oil is levorotatory. The low saponification and acid number value of essential oil indicated the presence of fewer amounts of fatty acids and free acids but high iodine value of the oil indicated the presence of more unsaturated compounds.

Table 2. *Physicochemical properties of the essential oil of A. Adenophora*

Parameters	Values
Specific gravity	0.947
Specific rotation	-37.3
Refractive index	1.549
Saponification value	24.4 Acid value 6.3
Iodine value	152

Antibacterial activity of essential oils of *A. adenophora* is shown in Table 3. Essential oil showed antibacterial activity against all four organisms tested. In comparison between the gram-positive and gram-negative bacteria, gram- positive bacterial strains were more susceptible to the concentration of oil than gram- negative bacteria suggesting that plant extracts are more active against gram-positive bacteria. Among the bacteria, *K. pneumonia* has higher value of 14 mm and *P. vulgaris* has lower value of 10 mm

in 1 mg/ml concentration but similar effect in both gram positive and gram negative bacteria at minimum concentrations of 0.25 mg/mL (Table 3). The present observation that *essential oil of A. adenophora* has antibacterial activity can be correlated with study done by Kurade et al. 2010[12]. Antibacterial effect of essential oils is, generally, less against gram-negative bacteria than gram-positive bacteria[13]. According to liteture survey, *A. adenophora* has not been used extensively for any purpose in Nepal. Therefore this plant may become a high risk posed invasive species in near future. Thus, here it is concluded that our finding will be supportive for using invasive alien species *A. adonophara* as an attractive source of bioactive natural products.

Table 3. *Antimicrobial activity of Ageratina adenophora essential oil against different micro-organisms*

	Zone of inhibition (ZOI) in mm/mL			
Concentration / Test organisms	0.25mg/mL	0.5mg/mL	0.75mg/mL	1mg/mL
E.coli	8	8	10	12
K. pneumoniae	8	8	9	14
P. vulgaris	8	9	10	10
S. aureus	8	8	10	12

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