ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

In vitro antioxidant, anti-acetylcholinesterase activity and GC-MS analysis of essential oil from flowers of *Ageratina adenophora* (Spreng)

S Vasanthi[1] and VK Gopalakrishnan[2]*

[1]Department of Biotechnology, Karpagam University, Coimbatore-641021, Tamilnadu, India
[2]*Department of Biochemistry and Bioinformatics, Karpagam University, Coimbatore-641021, Tamilnadu, India

ABSTRACT

In-vitro antioxidant, anti-acetylcholinesterase activity and GC-MS analysis of essential oil from flowers of *Ageratina adenophora* Spreng (Asteraceae) were determined. Their chemical composition was determined by hydro-distillation and analysed by GC-MS. The antioxidant activity was determined by DPPH, ABTS radical scavenging and Ferrous reducing antioxidant power (FRAP) assays. The essential oil was also evaluated for the anticholinesterase inhibition activity. Twenty five compounds were identified accounting for 91.3% of the oils. The essential oil was dominated by sesquiterpenoids (81.90) represented by sesuiterpene hydrocarbons (36.50) and oxygenated sesquiterpenoids (45.4%). The major compounds are copaen (19.72%), α-bisabolol (9.8%), 4,4-dimethyl-3-(3-methylbut-3-enylidine)-2-methylene bicycle [4.1.0] heptane(8'9%) and azulenone (9.5%). The IC_{50} value of the oil towards the antioxidant activity is 60.2 ± 2.3, 71.25 ± 1.5 µg/ml for the DPPH, ABTS radical scavenging assays respectively. Ascorbic acid is used as the standard substance and showed an IC_{50} value of 55.13 ± 1.2and 61.32 ± 2.6 µg/ml for DPPH and ABTS radical scavenging activity respectively. The oil showed good antioxidant activity by Ferrous reducing antioxidant power (FRAP) assay also. In the AChE activity the IC_{50} value of the oil and standard were determined as 96.25 ± 2.6 55.26 ± 0.96 respectively.

Keywords: *Ageratina adenophora* Spreng, Asteraceae, GC-MS, antioxidant, anti-acetylcholinesterase activity.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Ageratina adenophora (Spreng) (Syn. Eupatorium glandulosum) belongs to the Family Asteraceae is a profusely branching under shrub up to 90-120 cm in height with a few ascending branches; leaves simple, opposite, subsessile, lanceolate, subentire and glabrous type[1]. *A. adenophora* is a very common weed and invasion of this weed have replaced the larger part of the vegetation in through out our country and are considered as a major threat to native plants and animals [2-4]. It is reported to possess diverse medicinal properties and finds use in traditional medicines [5-11]. The leaves are used as astringent, thermogenic and stimulant in folklore medicine in India [1]. Presence of flavonoid glycosides in the leaves has been reported [12-13]. Many flavonol glycosides: 4'- methylquercetagetin 7-O-(6″-E-caffeoyl-β-d-glucoside) and quercetagetin 7-O-(6″-acetyl-β-d-glucoside), 7-*O*-glucosides of 6-hydroxykaempferol, quercetin and quercetagetin, 3,5,7-Trihydroxy-6,4'-dimethoxyflavone (betuletol and its 3-galactoside) have been isolated and characterized from the leaves of *A. Adenophora* [14, 15].

Despite the obnoxious nature and bad economic/environmental consequences the essential oil composition from the aerial parts of *A. adenophora* have been investigated earlier from different countries which report variety of terpenoid and other biologically active constituents from these plants [7-10]. However, information from the flower part is very meager and there is no report on the terpenoid composition of these aromatic weeds from India. This prompted us to carry out detailed chemical investigation of the essential oil from *A. adenophorum* Spreng for their possible utilization and having an overview on their aroma profile. From the fresh flowering aerial parts amorphenes (24.0%) were identified as the significant marker constituents of *A. adenophora* along with p-cymene (16.6%), bornyl acetate (15.6%), and camphene (8.9%). The amorphene derivatives identified in *A.adenophora* were amorph-4-en-7-ol (9.6%), 3-acetoxyamorpha-4,7(11)- dien-8-one (7.8%) and amorph-4,7(11)-dien-8-one (5.7%). Previously biologically active sesquiterpene lactones, cadinenes, chromenes and thymol derivatives were also detected [7-10, 17]. Earlier from our laboratory the antibacterial screening of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of *Eupatorium glandulosum* leaves exhibited a broad spectrum of inhibitory activity against Gram (+) and Gram (−) pathogenic bacteria[13].

MATERIALS AND METHODS

Plant Material

The botanical material (fresh flowering aerial part) *A. adenophorum* Spreng and was collected from the Western Ghats region Udhagamandalam, The Nilgiris, Tamilnadu
Plant herbarium and voucher specimens have been deposited and maintained in the Department.

Isolation of Essential Oils

The fresh flower (1 kg) was subjected to hydro-distillation using Clevenger-type apparatus for 3 hours. The oil was dried over anhydrous Na_2SO_4 and was stored in sealed vials under refrigeration prior to analysis. The oil yield was 0.40%.

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis of the oil was carried out on Shimadzu Model GC2014AF spectrometer fitted with Rtx-1 fused silica capillary column (30 m x 0.25 mm; 0.25 µm film coating). The column temperature was programmed from 60^0 C-90^0 C at 10^0 C /min, hold for 2 min, 90^0 C – 200^0 C at the rate of 5^0 C /min, hold for 2 min and finally 200^0 C – 250^0 C at the rate of 5^0 C /min and at 250^0 C hold for 5 min. using He as carrier gas at 1.0 mL/min. The injector temperature was 260^0 C, injection size 0.1µL prepared in *n*-hexane, split ratio 1:40. MS were taken at 70 eV with mass scan range of 40-450 amu. Identification of constituents were done on the basis of Retention Index, MS Library search (NIST and WILEY), by comparing with the MS literature data. The relative amounts of individual components were calculated based on GC peak area (FID response) without using correction factor.

Chemicals

Acetylthiocholine iodide (ATCI), Electric eel acetylcholinesterase and 5-5'-thiobis-2-nitrobenzoic acid (DTNB) were purchased from Sigma (USA). Eserine was obtained from Merck (Germany). All other reagents were of analytical grade. 2,2-Diphenyl-1-picrylhydrasyl (DPPH), 2,2'-azino-bis(3-ethylbenzothia zoline -6-sulphonic acid (ABTS), Potassium phosphate, L-ascorbic acid and gallic acid were purchased from HiMedia Laboratory , India. Potassium ferricyanide and ferric chloride were purchased from Qualigen Chemical Ltd., Mumbai, India. Other reagents were of analytical grade. Spectrophotometer (Shimadzhu UV-2450) was used to record absorbance.

Antioxidant Activity

DPPH radical scavenging activity

The antioxidant activity of the essential oil from the flower of *A. adenophora* (Spreng.) was measured in terms of hydrogen-donating or radical scavenging ability, using the Brand-Williams *et al.,* (1995) method[23] with a minor modification. Briefly, 1.5 ml of DPPH solution (10-4 M, in 95% Ethanol) was incubated with 1.5 ml of the essential oil separately at various concentrations (0.2-1.0 mg/ml). The reaction mixture was shaken well and incubated in the dark for 30 min at room temperature. The control was prepared as above without any extract. The absorbance of the solution was measured at 517 nm against a blank. The radical scavenging activity (RSA) was measured as a decrease in the absorbance of DPPH and was calculated. The assays were carried out in triplicate and the results were expressed as mean values ± standard deviation. The extract concentration providing 50% inhibition (EC50%) was calculated from the

graph of scavenging effect percentage against the extract concentration. Ascorbic acid was used as a standard.

Percentage of inhibition = [(Acontrol – Asample) / Acontrol] X 100%

Where A control was the absorbance of the control (blank, without extract) and
Asample was the absorbance in the presence of the extract or oil.

ABTS.+ radical scavenging activity

The essential oil from the flower of *A. adenophora* (Spreng.) was evaluated for its ABTS.+ radical scavenging activity as followed by Re *et al.,* (1999)[24] method with some modification. The experiments were carried out using an improved ABTS decolourisation method. ABTS.+ was generated by oxidation of ABTS.+ with potassium persulfate. 3ml of generated ABTS.+ solution were mixed with 30 ml solution the essential oil in different concentration like 0.2- 1.0 mg/ml. The decreasing of absorption was measured during 6 min at 734nm. The inhibition of the ABTS.+ radical scavenging assay calculated using the formula.

Percentage of inhibition = [(A control – A sample) / Acontrol] X 100%

Where Acontrol was the absorbance of the control (blank, without extract) and
Asample was the absorbance in the presence of the extract.

Ferrous reducing antioxidant Power (FRAP*)*

The ferrous reducing antioxidant power of the essential oil from the flower of *A. adenophora* (Spreng.) was measured according to the method of Oyaizu (1986)[25]. Various concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 mg/ml) of the extracts (0.5 ml) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%). The mixture was incubated at 500C for 20 min. TCA (10%: 2.5 ml) was added. The mixture was centrifuged at 650g for 10 min. The supernatant (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride and the absorbance was measured at 700 nm in a UV 2450 spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power. The ferrous reducing antioxidant power SSFE was compared with that of ascorbic acid (mg/ml).

Acetylcholinesterase activity assay

The AChE activity was measured according to the method developed by Ellman et al. [25]. This method employs ATCI as a synthetic substrate for AChE. In this procedure 10 µl of methanol extract containing 50 µg of crude extract was added to the reaction mixture containing 20 µl of enzyme solution (0.1 U/ml) and 950 µl sodium phosphate buffer (pH 8, 0.1 M). Reaction mixture was incubated for 15 min at 25 °C. Then, 10 µl of DTNB (10 mM) was added and the reaction initiated by the addition of substrate (10 µl of ATCI 14 mM solution). Based on this procedure, ATCI is broken down to thiocholine and acetate by the enzyme and thiocholine is reacted with DTNB to produce yellow color. The intensity of yellow color was

measured at 410 nm after 10 min. Eserine (20 µl of 0.1 mM solution in phosphate buffer) was used as positive control. The percentage of enzyme inhibition was calculated using the following formula.

$$\text{Inhibition\%} = 100-[A_t/A_c \times 100]$$

Where, A_t is the absorbance of the tested extract and A_c is the absorbance of the standard control.

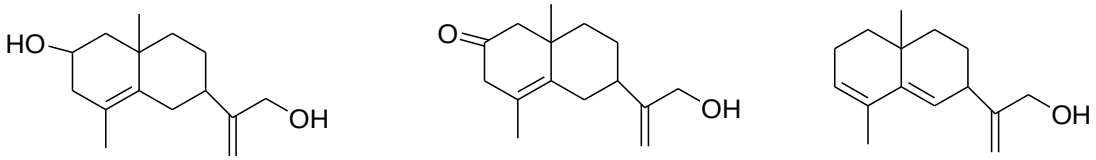
Statistical analysis

All experiments were repeated atleast thrice. The results were expressed as Mean ±Standard deviations.

RESULTS AND DISCUSSION

The identified constituents of the oils are listed in Table 1. A total of 25 compounds were identified accounting for 91.3% of the oils. The essential oil of was dominated by sesquiterpenoids (81.90%) represented by sesquiterpene hydrocarbons (36.50%) and oxygenated sesquiterpenoids (45.4%), with borneol acetate(5.9), germacrene D (8.1%), Copaen (19..72), α-Bisabolol (9.8%), 4,4-Dimethyl-3-(3-methylbut-3-enylidine)-2-methylene bicycle [4.1.0] heptane(8'9%) and Azulenone (9.5%) as major constituents. Four compounds were unidentified. Other constituents present in significant amount were monoterpenoids. In the present work the composition of the oil was different from the composition of the other oil obtained from other places. The flower essential oil of *A. adenophora* (Spreng.) of North Indian origin consists of 66% of monoterpenes and 28% of sesquiterpenes. Sixty-four constituents were identified and the amorphene derivatives (10%), were found to be main constituents of the sesquiterpene part. The presence of amorpha-4,7(11)-diene, 5,8-epoxyamorpha-3,7(11)-diene and amorpha-4,7-dien-11-ol, and muurol-4-en-7-ol, amorph-4-en-3,8-dione and muurol-4-en-3,8-dione ('eupatorenone') were also reported [22]. Amorphenes (24.0%) were identified as the significant marker constituents of *A. adenophora.*

In the present work the oil consists of compounds of the type **1**,**2** and **3** (Fig 1) which were identified for the first time from *A. adenophora* (Spreng.).



1 **2** **3**

Fig.1 Structures of the compounds 1, 2 and 3

ISSN: 0975-8585

Table 1. Essential oil composition of *E. adenophorum*.

S.No	Compound	Kovat index	% Composition	Molecular weight
1	Borneol	1138	0.76	196
2	Borneol acetate	1277	5.962	196
3	β-Caryophyllene	1494	1.13	204
4	Bergamotene	1430	0.62	204
5	β-Farnesene	1440	0.69	204
6	Germacrene D	1515	8.1	204
7	Elemene	1431	1.43	204
8	3,5,5-trimethyl-9-methylene-2,4a,5,6,7,8,9,9a-octahydro-1H-benzocycloheptene	1494	1.22	204
9	β-Sesquiphyllandrene	1446	0.87	204
10	Lanceol,Cis	1737	1.17	220
11	Hedycaryol	1694	3.1	222
12	Nerolidol	1564	1.14	222
13	Himachalene	1499	1.02	204
14	Germacrene D-4-ol	1660	1.25	222
	4,4-Dimethyl-3-(3-methylbut-3-enylidine)-2-methylene bicycle [4.1.0] heptane	1392	8.9	220
15	Copaen	1221	19.72	204
16	Ledene oxide (II)	1293	3.74	220
17	β-Guaiene	1523	1.70	204
18	α-Bisabolol	1625	9.8	222
19	Azulenone	1694	9.5	218
20	Cedren-13-ol	1646	0.68	220
21	1,5-dimethyl-3-hydroxy-8-(1-methylene-2-hydroxyethyl-1)-bicyclo[4.4.0] dec-5-ene	1933	2.92	236
22	6-(1,3-dimethyl-buta-1,3-dienyl)-1,5,5-trimethyl-7-oxa-bicyclo[4.1.0]hept-2-ene	1444	1.71	218
23	Compound I	1916	0.98	234
24	unidentified	1456	2.99	220
25	unidentified	1710	0.66	238
26	unidentified	1442	0.55	192
27	Compound 2	1727	0.93	218
28	unidentified	1558	1.21	218
29	Compound 3	1603	1.62	218

Acetylcholinesterase activity assay

The main role of acethylcholinestrase (AChE) is to rapidly hydrolyze acetylcholine at the cholinergic synapses, ending the transmission of nerve impulses. The use of AChE inhibitors in order to enhance cholinergic function in the brain is the main strategy in treatment of Alzheimer's disease (AD) which is characterized by loss or decline in memory and cognitive impairment. AD is the most common cause of dementia in the elderly and is responsible for 60 to 80 percent of the cases. Degeneration and loss of basal forebrain cholinergic innervation is accepted as a major cause of cognitive impairment and memory loss for the disease[24]. Several AChE inhibitors such as tacrine, donepzil, rivastigmine and galanthamine, are available

for the treatment of mild to moderate AD. Although the uses of these drugs are beneficial in the treatment of AD symptoms, they may also cause some adverse side effects. The most common side effects of these drugs include: anorexia, diarrhea, fatigue, nausea, muscle cramps as well as gastrointestinal, cardiorespiratory, genitourinary and sleep disturbances [25]. Therefore, cheaper and safer AChE inhibitors with higher efficacy, bioavailability and fewer side effects, particularly from natural sources, have been extensively investigated and research should be continued.

Nature is an unlimited resource for providing chemicals and biological compounds which are unique and complex in so far as their chemical synthesis seems impossible. The anti-cholinesterase activity of some plants in the world has been approved. In the present study we evaluated the essential oil from the flower of *A. adenophora* (Spreng.) for their anticholinesterase activity. IC_{50} values were calculated as 96.25 ± 2.6, 55.26 ± 0.96 essential oil (Fig.2) and standard from the regression equation obtained from various concentrations of the test compounds.

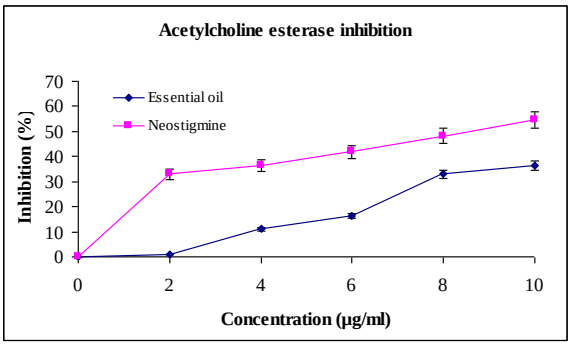


Fig.2 The anticholinesterase inhibition activity of the essential oil from *A.adenophorum*

Results of this study indicated that these plants may offer great potentials for the treatment of different diseases including AD, and their anti-acetylcholinesterase properties introduce them as promising candidates for more detailed *in vitro* and *in vivo* studies. Besides, these plants can be examined in order to isolate and identify the active ingredients, and this may serve as a foundation to find safer and more effective agent (s) for therapeutic use.

Antioxidant Activity

It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health benefical effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects, and herbivores[19]. Several polyphenolic compounds were already reported from *A. adenophora* (Spreng.) *extract* and it may be of great benefit in understanding the health aspects of both the traditional and modern uses of this plant.

ISSN: 0975-8585

Several assays have been frequently used to estimate antioxidant capacities in fresh fruits and vegetables and their products and foods for clinical studies including 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS.+)[20], 2,2- diphenyl-1-picrylhydrazyl (DPPH.)[30], ferric reducing antioxidant power (FRAP)[21]. The results of the present study are shown in the Figure 3-5 illustrates the antioxidant activity by DPPH radical scavenging method, ABTS.+ and FRAP method at various concentration of the essential oil of *A. adenophora* (Spreng.).

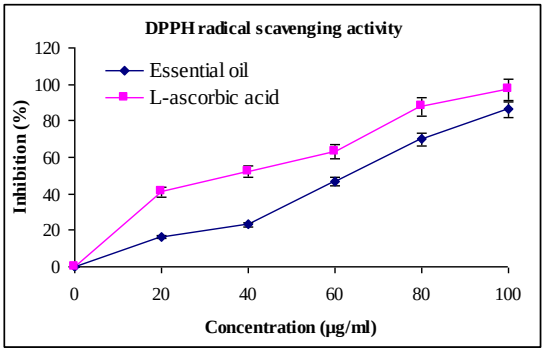


Fig.3 Antioxidant activity of the essential oil by DPPH method

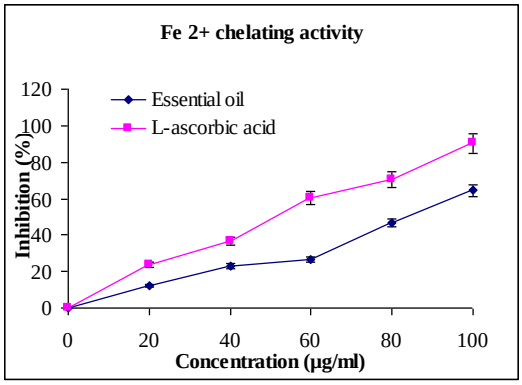


Fig.4 Antioxidant activity of the essential oil by ABTS•+ assay

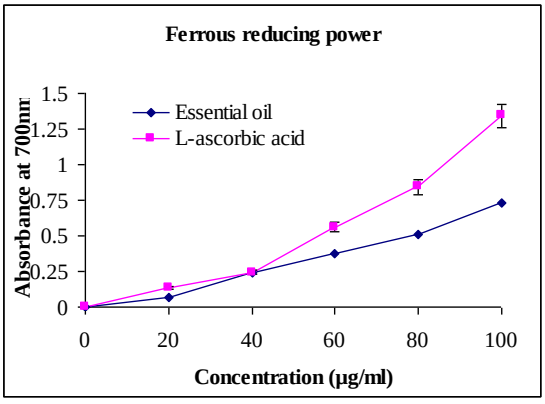


Fig.5 The ferrous reducing antioxidant power of the essential oil

The IC_{50} value of antioxidant activity of the essential oil is found to be 60.2 ± 2.3 µg/ml in standard (L-ascorbic acid), 55.13 ± 1.2 µg/ml respectively by DPPH. (Fig. 3). The ABTS.+ scavenging activity found to be good with an IC_{50} value 71.25 ± 1.5, 61.32 ± 2.6 µg/ml respectively (Fig. 4). The ABTS•+ assay is a decolourization method, the radical formed before the addition of the sample is in a stable form and blue in colour and after addition of the sample the ABTS•+ solution decolorises. The ferrous reducing antioxidant power of the essential oil measured by the direct reduction of Fe[(CN6)]3 to Fe[(CN6)], the addition of free Fe3+to the reduced product leads to the formation of the intense perl's Prussian blue complex, Fe4[Fe(CN-)6]3, which has strong absorbance at 700 nm[22] (Fig. 5). The increasing absorbance of the reaction mixture indicating the high reducing antioxidant power of the fruit extract.

In a recent study The antioxidant activities of the oil and cadinenes were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the ferric reducing ability assay (FRAP), together with three antioxidant standards, i.e. ascorbic acid, tert-butyl-4-hydroxy toluene (BHT), and gallic acid. The antioxidant activity of the essential oil was comparable with the standards. The essential oil and the cadinene sesquiterpene-rich extract of *E. adenophorum* have exhibit potential antioxidant activities. Wide availability of the plant and the potential antioxidant activity makes it a potential natural antioxidant.

CONCLUSION

In order to make use of a harmful weed, *A.adenophorum* Spreng., its odorous constituents were studied. Its aroma quality and possible application were evaluated. 29 compounds were identified qualitatively and quantitatively in the essential oil by GC / MS, the main compounds were with borneol acetate(5.9), germacrene D (8.1%), Copaen (19..72), α-Bisabolol (9.8%), 4,4-Dimethyl-3-(3-methylbut-3-enylidine)-2-methylene bicycle [4.1.0] heptane(8.9 %) and Azulenone (9.5%) as major constituents. The essential oil of *A. adenophorum* has exhibited potential antioxidant activities. Wide availability of the plant and the potential antioxidant activity makes it a potential natural antioxidant. Results of anti-acetylcholinesterase study indicated that *A. adenophorum* may offer great potentials for the treatment of different diseases including AD, and their anti-acetylcholinesterase properties and suggests for more detailed *in vitro* and *in vivo* studies. Besides, this may serve as a foundation to find safer and more effective agent (s) for therapeutic use. The results shown that the weed is a kind of valuable raw perfumery material. The application of the weed to the perfume Industry will result in economic profits but also control the spread of the harmful weed.

ACKNOWLEDGEMENT

We, the authors are thankful to Chancellor, Chief Executive Officer, Vice-Chancellor and Registrar of Karpagam University for providing facilities and encouragement.

REFERENCES

[1] Kritikar KR, Basu BD. Indian MedicinalPlants, Bishen Sing and Mahendra Pal Singh: Derhadun, India 1987; 3: 1331-1333, 1977-1978.
[2] Mukherjee PK, Mukherjee K, Hermans-Lokkerbol1 ACJ, Verpoorte R, Suresh B. J Nat Rem 2001; 1(1): 21 – 24.
[3] Ansari S, Jain P, Tyagi RP, Joshi BC. Barar SK. Herba Polon 1983; 29: 93-96.
[4] Mandal SK, Mandal SC, Das AK, Tag H, Sur T. Indian J Nat Prod 1981; 21: 6-8.
[5] Bardoli MJ, Shukla VS, Sharma RP. Tetrahedron Lett 1985; 26; 509-510.
[6] Baruah NC, Sarma JC, Sarma S, Sharma RP. J Chem Ecol 1994; 20:1885-1895.
[7] Adhikari R, Kraus W. J Nepal Chem Soc 1994; 13:34-41.
[8] Ding JK, Wanf P, Yu XJ, Yi YF, Ding ZH. Chem Abstr 1982; 117: 556-558.
[9] Weyerstahl P, Marschall H, Seelmann I, Kaul VK. Flav Fragr J 1997; 12: 387-397.
[10] Padalia RC, Bisht DS, Joshi SC, Mathela CS. J Essent Oil Res 2009; 21**:** 522-524.
[11] Sasikumar J.M, Pichai Anthoni Doss A, Doss A. Fitoterapia 2005; 76(2): 240–24
[12] Nair AGR, Jayaprakashan R, Gunasekharan R, Bayet C, Voirin B. Phytochem 1993;33:1275-76.
[13] Nair AGR, Gunasekharan R, Krishnan S, Bayet C, Voirin B. Phytochem 1995; 40: 283-285.
[14] Ramachandran Nair A.G, Gunasegaran R, Sujatha Krishnan, Christine Bayet
[15] Bernard Voirin. Photochem 1995; 40(1): 283–28
[16] Ramachandran Nair AG, Sivakumar R. Phytochem, 1990; 29(3):1011-1012.
[17] Bohlmann F, Gupta RK. Phytochem 1981; 20: 1432-1433.
[18] Pala-Paul J, Perez-Alonso MJ, Velasco-Negueruela A, Sanz J. J Chromatogr A 2002; 947: 327–331
[19] Aditi Kundu, Supradip Saha, Suresh Walia, Vivek Ahluwalia and Charanjeet Kaur. Toxicol Environm Chem 2013; 95(1): 127-137
[20] Vaya J, Belinky PA, Aviram M. Free Radical Biol Med 1997; 23(2): 302-313.
[21] Leong LP, Shui G. Food Chem 2002; 76: 69–75.
[22] Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. Nutr Res 2003: 23: 1719–1726.
[23] Ilhami G. Chem-Biol Interact 2009; 179: 71-80.
[24] Mukherjee PK, Kumar V, Mal M, Houghton PJ. Phytomed 2007;14:289–300.
[25] Ellman GL, Courtney KD, Andres V, Feather-Stone RM. Biochem Pharmacol 1961;7:88–95.