Journal of Chemical, Biological and Physical Sciences

An International Peer Review E-3 Journal of Sciences

Available online at www.jcbsc.org

Section B: Biological Sciences

CODEN (USA): JCBPAT Research Article

Chemical Composition and Antioxidant Activity of *Citrus Medica Var Acidica* Essential Oil

[*]Swadesh Malhotra [1], Ruchi Shukla [1], Shweta Kulshrestha [1] and Aisha Siddiqui [2]

[1] Phytochemistry Division, CSIR-National Botanical Research Institute Rana Pratap marg, Lucknow, UP, India.

[2] Department of Chemistry, Integral university, Kursi road lucknow, UP, India.

Received: 01 April 2016**; Revised:** 22 April 2016**; Accepted:** 26 April 2016

Abstract: *Citrus* commonly known as lemon is well established for its pharmaceutical and economic importance. The volatile compounds of peel of *C.medica var acidica* (Rutaceae) were analysed using GC, GC–MS. Analysis of the oil resulted in the identification of 20 compounds, representing 95.17% of the oil. The major compounds of the oil are d-limonene (9.66%), geraniol (24.56%), geranial (31.54%), neral acetate (9.33%), geranyl acetate (6.2%). The samples viz. essential oil (A), it's pure constituents geraniol (B) and geranial (C) were subjected to a screening for its possible antioxidant activity by using β-carotene-linoleic acid assays. It was found that the total essential oil (A), its pure constituent's geraniol (B) and geranial (C) have marked to significant antioxidant activity. In β-carotene-linoleic acid system, C. medica essential oil (A), geraniol (B) and geranial (C) exhibited -59.64 ± 1%, 24.56 % and 31.56% antioxidant activity.

Keywords: *Citrus medica var acidica*, essential oil, chemical composition, geraniol, geranial, antioxidant activity.

INTRODUCTION

Citrus medica var *acidica* (Rutaceae) is found in the base region of Himalya, from Gadwal to Sikkim at the height of 4000 feet. It is also seen in Assam, central India and Western Ghats of India. *Citrus,*commonly known as lemon is well established for its pharmaceutical and economic importance. Lemon is used for the treatments of fever, anxiety, soar throat, blood pressure, diarrhea, arthritis, gall stones, digestive problems and nervous conditions.It is used as a tonic, astringent, antiseptic, laxative and tones the liver. The essential oils from the genus *Citrus* are natural flavoring materials of commercial importance and in India, local people use it as a good source of fresh juice. Its juice is used mostly in digestion related problems, also given in conditions like nausea, emesis, piles and wormal infestation and acts in stimulating liver for proper secretion of bile juices. It is also used in controlling cough, cold and asthma, menstrual disturbances. It is very widely used in rehabilitating alcoholics and drinking habits. Its leaves are externally used in case of pains and inflammation on the external body part, skin disorders and paste of seeds is also very helpful in scorpion bite [1, 2].

Citrus medica L. cv. *Diamante* (Diamante citron) peel extract showed hypoglycaemic activity and an anticholinesterase effect [3]. Gao *et al* have reported limettin, stigmasta-5, 22-dien from *Citrus medica L.var. sarcodactylis* [4]. Previous reports of different cultivars of *C.medica* var acidica oil indicate varying concentration of citronellal (27.5 to 63 %), citronellol (13.0 to 15.1%) and limonene (8 to 55.5%), apart from other compounds [5] .In vitro, the antioxidant activities of monoterpenes and diterpenes have been reported earlier and terpenoids/ isoprenoids like α, β, δ-carotenes, lycopene, astaxanthine, lutein and zeaxanthene are well known antioxidants. Monoterpenes help to stimulate the levels of liver enzymes that aid in detoxifying carcinogens and trigger apoptosis and considered to be responsible for the health promoting properties of fruits and vegetables [6]. *Citrus* limonoids possess antitumour, anticarcinogenic and antifeedant activity against insects and show synergestic effect in prevention of certain types of cancers and cardiovascular diseases [7]. *Citrus* peel, a by product of the juice industry, possesses detergent constituents, is used as antimicrobial agent [1] and is rich source of essential oils and flavonoids. It has been investigated as a new source of antioxidant, in terms of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, β-carotene bleaching and nitrite scavenging activities [8-12].

Citrus peel is a byproduct of the juice industry and has been investigated for its antioxidant activity, in terms of β-carotene bleaching activity [1, 9]. It have been reported as a major source of dietary d-limonene, with a potential protective effect in relation to squamous cell carcinoma of the skin [13]. The aim of the present study was to evaluate the in vitro antioxidant ability of the volatile component of *Citrus medica* var acidica.In vitro, antioxidant activity was determined by using β-carotene linoleic acid system. The chemical composition of the essential oil was evaluated by using GC, retention indices (RI) and GC-MS [14-16].

MATERIALS AND METHODS

Plant Material: Fruits of *C.medica* var *acidica* were collected from Lucknow (UP, India), in season Nov–Jan. The plant material (voucher specimen no. 227540) was identified by the taxonomic department of National Botanical Research Institute, Lucknow, India. The same has been deposited to the herbarium of the institute. The fresh peels were separated from the fruit for present studies.

Instruments

a) GC-Perkin Elmer Autosystem XL gas chromatograph equipped with column PE-5 (50m X 0.32mm X 0.25µm/4 film), Norwalk CT, USA.

b) GC/MS Perkin Elmer turbo mass coupled with GC-AutoXL, Norwalk CT, USA, mass selective detector in the electron impact mode (70 eV).

c) Varian Cary 50 Bio UV Visible spectrophotometer.

Chemicals and Reagents: The linoleic acid and β-carotene were purchased from Acros, USA, Tween 40 (Sigma- Aldrich Co., USA), Methanol and chloroform (HPLC grade) and other analytical grade chemicals from E. Merck, India. α and β-Pinene, geraniol, geranial, geranyl acetate, neral acetate were procured from Flavour and Fragrance Development Center, Kannouj, India. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, the NIST98 and Wiley275 library data and literature. Relative RI were calculated using a GC data of a homologous series of saturated aliphatic hydrocarbons within C8 to C22, performed at the same column and conditions as used in the GC analysis for the essential oils [12, 17, 18].

Extraction of Essential Oil Hydrodistillation Method: The fruit peels of *C.medica* var *acidica* (100g) were crushed and subjected to hydrodistillation for 4 hrs using Clevenger's apparatus [19]. The oil obtained (yield 1.1%) was dried over anhydrous sodium sulphate and preserved at -20˚C temperature.

GCMS Studies of Essential Oil

a) GC analysis was performed on a GC-Perkin Elmer Autosystem XL gas chromatograph equipped with column PE-5 (50m X 0.32mm X 0.25µm/4 film), Norwalk CT, USA. Injector and detector temperatures were set at 100 and 280˚C, respectively. The oven temperature was held at 50˚C for 3 min, then programmed to 280˚C at a rate of 3˚C /min. Helium was the carrier gas at a flow rate of 1.26 ml/min and with inlet pressure 10 psi. Turbochrom software was used for the determination of the percentage of components.

b) GC–MS analysis of the essential oil was performed under the same conditions with GC (column, oven temperature, flow rate of the carrier gas) using a GC/MS Perkin Elmer turbo mass coupled with GC-AutoXL, Norwalk CT, USA, mass selective detector in the electron impact mode (70 eV).

Antioxidant Activity by β-carotene Bleaching Assay: Antioxidant activity of *Citrus* essential oils and synthetic antioxidant (BHT) were determined by the method of β-carotene bleaching [12, 20, 21]. 1 mg of β-Carotene was dissolved in 40 ml of chloroform (HPLC grade). This solution (3mL) was added to 40 mg linoleic acid and 400 mg Tween 40. Chloroform was removed under vacuum and aerated distilled water (100 mL) was added to the β-carotene emulsion with vigorous shaking. Aliquots (6 mL) of this emulsion were transferred into test tubes containing essential oils and the absorbance was measured at 470 nm using a UV visible spectrophotometer, at zero time. The tubes were incubated at 50˚C in a water bath for 60 min and absorbance was again measured at 470 nm. Methanol (HPLC grade) was used instead of essential oil as a control treatment. BHT was used as standard for comparative studies.

Degradation rate of extracts was calculated according to the following equation:

Sample degradation rate = ln (a/b) X 1/t

Where, ln = natural log, a = initial absorbance at time 0, b = absorbance at 60 min, t = incubation time (min).

Antioxidant activity (AOA) was expressed as percentage inhibition relative to the control using the following equation:

$$\text{AOA} = \frac{\text{Degradation rate of control - Degradation rate of sample}}{\text{Degradation rate of control.}} \times 100$$

RESULTS

The chemical compositions of the essential oils from *C. medica* var *acidica* have been shown in **Table-1**. It was observed that the fruit peel of *C. medica* var *acidica* is a very rich in geraniol (24.56%), geranial (31.54%),neral acetate (9.33%), geranyl acetate (6.23%) with a lesser amount of d-limonene (9.66%). **Figure-1** shows the antioxidant activity of the *Citrus medica* essential oils geranial and geraniol. This assay is based on the radical adducts of carotenoid with free radicals from linoleic acid.

The linoleic acid free radical attacks the highly unsaturated β-carotene models. The presence of different antioxidants can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system [12, 20]. It has been observed that oil under investigation has significant activity (59.6%)) while its pure constituents geraniol and geranial showed a decreased activity (24.5% and 31.5%) respectively **Figure-1**.

Table-1: Chemical composition of essential oil of *C. medica.*

S. No.	Compound	%	RI
1.	α-pinene	0.91	937
2.	b- pinene	0.56	979
3.	Myrcene	1.80	997
4.	Sabinene	2.17	965
5.	D-limonene	9.66	1028
6.	α-phellendrene	0.05	999
7.	Terpine-4-ol	1.16	1169
8.	Citronellal	2.88	1154
9.	Dodecanal	0.51	1388
10.	Nerol	0.46	1225
11.	Neral	0.27	1238
12.	Geraniol	24.56	1274
13.	Geranial	31.54	1267

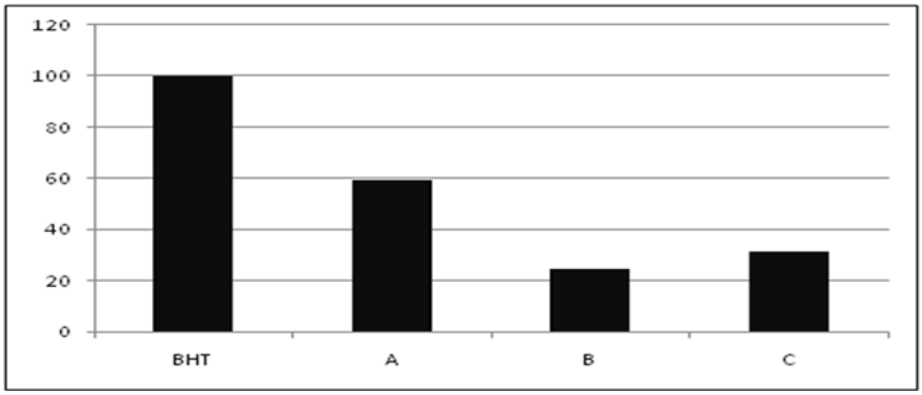

Figure 1: Antioxidant activity of C. medica essential oil **(A)** Geraniol **(B)** Geranial.

DISCUSSION

Monoterpenes and diterpenes which are the main components of essential oils. Their antioxidative capacity is believed to be responsible for the health promoting properties of fruits and vegetables. Several investigations have studied the antioxidant activity of monoterpenes and diterpenes or essential oils in vitro [22]. Previous studies have reported chemical composition and antioxidant activity of essential oil of Citrus cultivars and limonene [12]. The present investigations also support the previous work [12, 23] confirming that the monoterpene hydrocarbons show antioxidant activity. The antioxidant activity of essential oil of *C.medica* var *acidica* and its major constituents viz. geranial and geraniol have been carried out by β-carotene bleaching method. This assay is based on the radical adducts of carotenoid with free radicals from linoleic acid. The linoleic acid free radical attacks the highly unsaturated β-carotene models.

The presence of different antioxidants can hinder the extent of β-carotene-bleaching by neutralizing the linoleate-free radical and other free radicals formed in the system [20, 21] .The antioxidant activity of Citrus samples decreased in the order- BHT > *C.medica* var *acidica* > geranial > geraniol. Inhibition of free radical–induced damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of diseases [24]. The synthetic antioxidants like butylated hydroxyanisole and butylated hydroxytoluene, commonly used as preservatives in pharmaceutical, cosmetic, and food products, allegedly cause liver damage and carcinogenesis in laboratory animals [14]. During last decades, there has been considerable interest in the use of naturally occurring antioxidants for treatment or prophylaxis of various oxidative stress-related diseases [15]. Terpenoids/ isoprenoids like α, β, γ carotenes, lycopene, astaxanthine, lutein and zeaxanthene are well known antioxidants. Monoterpenes help stimulate the levels of liver enzymes that aid in detoxifying carcinogens and trigger apoptosis. The peel of the *Citrus* fruit contains the highest concentrations of flavonoids and essential oils [7-9, 23-25] have studied antioxidant behaviour of essential oils in Citrus species .In the present studies the fruit peel of *C. medica* var *acidica* , which is an agro waste , has been identified as a rich source of geraniol (24.56%), geranial (31.54%),neral acetate (9.33%), geranyl acetate (6.23%) and contains lesser amount of d-limonene (9.66%). It can be used in food , nutraceutical and cosemaceutical industries , as oil under investigation has significant antioxidant activity (59.6%)) while its pure constituents geraniol and geranial showed a decreased activity (24.5% and 31.5%) respectively.

ACKNOWLEDGEMENT

The authors are thankful to Director National Botanical Research Institute, Lucknow India for his kind support and Central Institute of Medicinal and Aromatic Plants, Lucknow, India for providing instrumentation facilities.

REFERENCES

1. L.V. Asolkar, K.K. Kakkar, O.L. hakre. Second supplement to Glossary of Indian Medicinal *Plants*, Publication & Information Directorate, (CSIR), New Delhi. 1992, part I: 210-11.
2. I.S. Young, J.V. Woodside. Antioxidants in health and disease. *J. Clin. Pathol*. 2001, **54**: 176–186.
3. F. Conforti, G.A. Statti, R. Tundis, M.R. Loizzo, F. Menichini. *Phytother Res.* 2007, **21** (5*)*: 427-33.
4. Y. Gao, H. Huang, H. Xu, Y. Diao, Z. Dong. Studies on the chemical constituents of Citrus medica var. sarcodactylis. *Planta Medica*. 2009, **75** (1): 62-64.
5. G. Singh, O.P. Singh. Chemistry of Essential oils of *Citrus* species. *Natural Product Radiance*. 2002, **1**: 8-21.
6. B. Patil, G.K. Jayaprakasha, E.D. Harris. Impact of *Citrus* limonoids on human health. *Acta Horticulturae*. 2007, **744**: 127-34.
7. J.Y.F. Sun, X. Chu, R. Wu, H. Liu. Antioxidant and antiproliferative activities of common fruits. *J Agric Food Chem*. 2002, **50**: 7449-7454.
8. M.A. Anagnostopoulou, P. Kefalas, E. Kokkalou, A.N. Assimopoulou, V.P. Papageorgiou. Analysis of antioxidant compounds in sweet orange peel by HPLC-DAD-MS (ESI+). *Biomedi chromatog*. 2005, **19**: 138-148.
9. M.A. Anagnostopoulou, P. Kefalas, V.P. Papageorgiou, A.N. Assimopo ulou, D. Boskou. Radical scavenging activity of various extracts and fractions of sweet orange peel (*C.sinensis*). *F. Chem.* 2006, **94** (1): 19-25.
10. W. Droge. Free radicals in the physiological control of cell function. *Physiol Rev.* 2002, **82**: 47–95.
11. S. Gorinstein, O.M. Belloso, Y. Park, R. Haruenkit, A. Lojek, M. Ciz, A. Capsi, I. Libman, S. Trakhtenberg. Comparison of some biochemical characteristics of different Citrus fruits. *Food Chem*. 2001, **74**: 309–315.
12. S. Malhotra, S. Suri, R. Tuli. Antioxidant activity of Citrus cultivars and chemical composition of *Citrus karna* essential oil. *Planta Medica*. 2009, **75** (1*)*: 62-65.

13. I.A. Hakim, B. Harris, C. Ritenbaugh. *Citrus* peel use is associated with reduced risk of squamous cell carcinoma of the skin. *Nutrition and Cancer*. 2000, **37**:161-8.

14. D.L. Madhav, D.K. Salunkhe. Toxicological aspects of food antioxidants. In Food Antioxidants, Marcel Dekker, New York. 1995, 267.

15. S.R.J. Maxwell. Prospects for the use of antioxidant therapies. *Drugs*. 1995, **49**: 345–361.

16. N.J. Miller, C.A. Rice-Evans. The relative contributions of ascorbic acid and phenolic antioxidants to the total antioxidant activity of orange and apple fruit juices and blackcurrant drink. *F. Chem*. 1997, **60** (3): 331–337.

17. W. Jennings, T. Shibamato. Qualitative Analysis of Flavor and Fragrance Volatiles by Glass Capillary Gas Chromatograph. Ist Ed. Academic Press, New York. 1980, 9-10.

18. S. Malhotra, R. Banerjee, A.K. Gupta, S.M. Jain. Seasonal variation in volatile content of *Citrus sinensis*. *Indian Perfumer*. 2007, **51** (3): 35-6.

19. J. Clevenger. Apparatus for the determination of volatile oil. *J Am Pharm Assoc.* 1928, **17**: 345-9.

20. G.K. Jayaprakakasha, T. Selvi, K.K. Sakariah. Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Research International*. 2003, **36**: 117-22.

21. C.L. Emmons, D.M. Peterson. Antioxidant activity and phenolic contents of oats, groats and hulls. *Cereal Chem*. 1999, **76**: 902-906.

22. J. Grassmann. Terpenoids as plant antioxidants. *Vitam Horm*. 2005, **72**: 505-35.

23. B. Tepe, A.S.. Tepe, D.M. Daferera, M. Polissiou, A. Sokmen. Chemical composition and antioxidant activity of the essential oil of *Clinopodium vulgare L*. *Food Chem.* 2007, **103**: 766– 70.

24. D.E. Brash, P.A. Harve. New careers for antioxidants. *Proc Natl Acad Sci*. USA. 2002, **99**: 13969–13971.

25. J.A. Manthley, K. Grohmann. Phenols in citrus peel byproducts. Concentrations of hydroxycinnamates and polymethoxylated flavones in citrus peel molasses. *J Agri Food Chem*. 2000, **149**: 3268–3273.

***Corresponding author: Swadesh Malhotra;**

Phytochemistry Division, National Botanical Research Institutedia, Rana Pratap Marg, Lucknow, UP, India.