African Journal of Biochemistry Research Vol. 4(7), pp. 191-195, July 2010
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 ©2010 Academic Journals

Full Length Research Paper

Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*

P. Praveen Kumar[1]*, S. Kumaravel[1] and C. Lalitha[2]

[1]Food Testing Laboratory, Indian Institute of Crop Processing Technology, Pudukkottai Road, Thanjavur-613 005. Tamil Nadu, India.
[2]Department of Biochemistry, Dhanalkshmi Srinivasan Arts and Science College for Women, Perambalur-621212, Tamil Nadu, India.

Accepted 11 June, 2010

The present study was carried out for identification of the phytochemicals present in the *Vitex negundo* leaves and also evaluate the total phenols, total flavonoids and antioxidant activity of the leaf extract. Total phenols was carried out by Folin Ciocalteu method and the phenolic content was 27.72 mg/100 of gallic acid equivalent (GE). Antioxidant activity was evaluated by DPPH method and the leaves of *V. negundo* showed 23.21 mg/100 of Ascorbic acid Equivalent Antioxidant Capacity (AEAC). The GC-MS study also carried out and it showed the presence of phytochemicals like 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-(RT:6.17), Phytol (RT:19.67) and Vitamin E (RT:25.11).

Key words: Total Phenols, total flavonoids, antioxidant activity, DPPH, GC-MS.

INTRODUCTION

Vitex negundo belongs to the family verbenaceae is a large aromatic shrub or a small tree of about 3M in height (Kirtikar and Basu, 1976). *V. negundo* leaves may have both central and peripheral analgesic action and also possesses antiinflammatory activity by acting through inhibition of prostaglandin biosynthesis (Telang et al., 1999). The mature fresh leaves of *V. negundo* have oral anti-inflammatory, analgesic and antihistamine properties (Dharmasiri et al., 2003).

Oxygen derived free radicals and their products are known to play an important role in the pathogenesis of chronic inflammatory disorders (Blake et al., 1981). *V. negundo* contains many polyphenolic compounds, terpenoids, glycosidic iridoids and alkaloids. Since polyphenolic compounds have high antioxidant potential, the antioxidant potency of *V. negundo* was investigated by employing various established *in vitro* systems (Om Prakash et al., 2007). The present study was carried out to study the flavonoids, phenols and antioxidant activity of *V. negundo* and the chemical constituents were studied by GC-MS.

*Corresponding author. E-mail: pravee.21msc@gmail.com. Tel: 09486753346.

MATERIALS AND METHODS

Collection and processing of plant material

The leaves of the plant *V. negundo* collected from Thanjavur District in the month of January, 2009 and authenticated by Dr. John Britto, Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. The leaves were cleansed and shade dried for a week and grounded into uniform powder. 1 g of plant material was added to 20 ml of aqueous methanol (20%, v/v) for 18 h at room temperature. The extracts were filtered and used for the estimation of total phenols and antioxidant activity.

Total phenols

0.5 ml of freshly prepared sample was taken and diluted with 8 ml of distilled water. 0.5 ml of Folin Ciocalteu Reagent (1 N) was added and kept at 40˚C for 10 min. 1 ml of Sodium Carbonate (20%) was added and kept in dark for one hour. The color was read at 650 nm using Shimadzu UV-1650 Spectrophotmeter (Malick et al., 1980). The same procedure was repeated for all standard gallic acid solutions and standard curve obtained. The sample concentration was calculated as Gallic acid equivalent (GE).

Total flavonoids

0.5 ml of aqueous extract of sample is diluted with 3.5 ml of distilled water at zero time and 0.3 ml of 5% Sodium Nitrate was added to

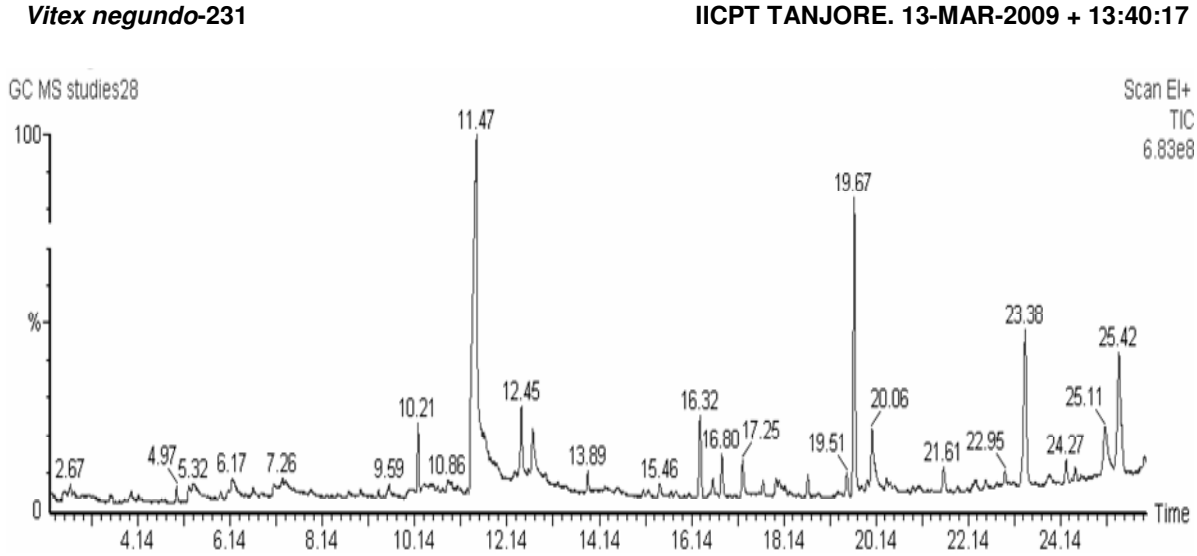

Figure 1. Chromatogram of *V. negundo* leaves by GC-MS.

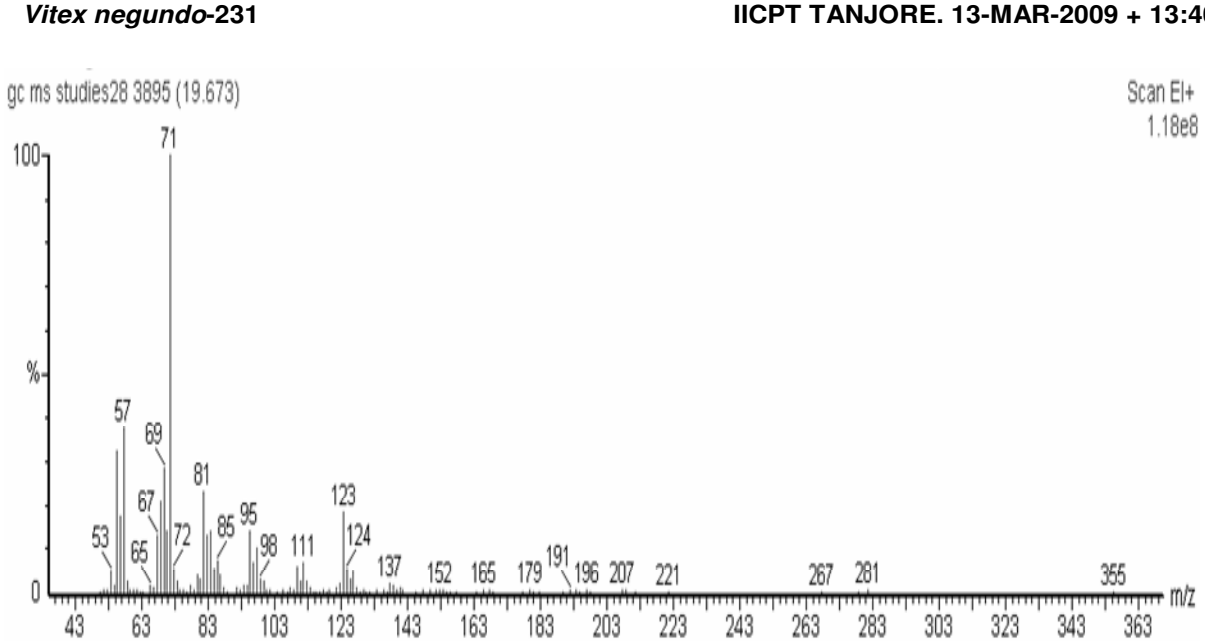

Figure 2. Mass spectrum of Benzoic acid, 3-hydroxy-

the tubes. After 5 min, 0.3 ml of Aluminium Chloride (10%) was added to all the tubes. At the 6th minute, 2 ml of Sodium Hydroxide (1 M) was added to the mixture. Immediately, the contents of the reaction mixture were diluted with 2.4 ml of distilled water and mixed thoroughly. Abosorbance of the mixture was determined at 510 nm versus a prepared blank immediately. Gallic acid was used as the standard compound for quantification of total flavonoids as mg/100 g (Zhisen et al., 1999).

Antioxidant activity

DPPH method

0.1 ml of the freshly prepared sample was taken in test tubes. 6 ml of DPPH solution (0.1 mM) was added and the tubes kept in dark for one hour. The color was read at 517 nm. The difference in the O.D of DPPH solution and DPPH solution + sample was calculated.

Table 1. Total phenols, flavonoids and antioxidant activity in the leaves of *V. negundo.*

S/No.	Parameter analysed	Values obtained
1	Total phenols (mg/100 g) GE*	27.72 ± 0.3
2	Total flavonoids (mg/100 g) GE*	196.04 ± 0.8
3.	Antioxidant activity (mg/100 g) AEAC**	23.21 ± 0.96

The values are mean value of three replicates. * - Gallic acid equivalent, ** - Ascorbic acid equivalent antioxidant capacity.

The decrease in OD with sample addition is used for calculation of the antioxidant activity. Ascorbic acid standards were prepared in different concentrations and antioxidant was determined as ascorbic acid equivalent antioxidant capacity (AEAC) mg/100 g of sample (Koleva et al., 2002).

GC –MS analysis

Preparation of extract

Leaves of *V. negundo* were shade dried. 20 g of the powdered leaves were soaked in 95% ethanol for 12 h. The extracts were then filtered through Whatmann filter paper No.41 along with 2 gm sodium sulfate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with 95% ethanol. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract contained both polar and non-polar phytocomponents of the plant material used. 2 µl of these solutions was employed for GC/MS analysis (Merlin et al., 2009).

GC analysis

GC-MS analysis was carried out on a GC clarus 500 Perkin Elmer system comprising a AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-1 fused silica capillary column (30 × 0.25 mm ID ×1EM df, composed of 100% Dimethyl poly siloxane), operating in electron impact mode at 70 eV; helium (99.999%) was used as carrier gas at a constant flow of 1ml/min and an injection volume of 0.5 EI was employed (split ratio of10:1) injector temperature 250°C; ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da.

Identification of components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS AND DISCUSSION

Total phenolics and flavonoid content in the leaves of *V. negundo*

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003). Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Om Prakash et al., 2007). The flavonoid contents of the extracts in terms of gallic acid equivalent (Table 1). Total phenolic content of the methanolic extract of *V. negundo* leaves is 27.72 mg/100 g of GE. The highest value of phenolic content indicates that the plant has high antioxidant activity.

GC-MS Study

The GC-MS study of *V. negundo* leaves has shown many phytochemicals which contributes to the medicinal activity of the plant (Tables 2 and 3). The major components which present in the leaves of the plant *V. negundo* was Benzoic acid 3-hydroxy (RT:11.47) (Figure 2), Ledol (RT:12.45), 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- (RT:23.38) and Vitamin E. The other components like 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (RT: 6.17), Caryophyllene (RT: 10.21) and n-Hexadecanoic acid also present in the leaves of *V. negundo* (Figure 1).

Conclusion

The study clearly indicates that the leaf extract was rich in antioxidants, phenolics and flavonoids. The GC-MS study also showed many Phytochemicals Hexanoic acid, ethyl ester, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, Hexadecanoic acid, ethyl ester, Caryophyllene, Benzoic acid, 3-hydroxy-, Ledol, Aromadendrene oxide-(1), n-Hexadecaonoic acid, Phytol, 9,12,15-Octadecatrienoic acid, (Z,Z,Z)- and Vitamin E which contributes the activities like antimicrobial, antioxidant anticancer, Hypercholesterolemic, Antiulcerogenic and

Table 2. Phytocomponents identified in the methanolic extract of the leaves of *V. negundo* by GC-MS.

RT	Name of the compound	Peak area (%)
2.67	d-Mannose	0.24
3.53	Butane, 1,1-diethoxy-3-methyl	0.06
3.98	Hexanoic acid, ethyl ester	0.22
4.97	Propane, 1,1,3-triethoxy-	0.12
5.32	2,3-Dihydrothiophene 1,1-dioxide	0.67
6.17	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	0.55
7.26	2,4-Pentadien-1-ol, 3-propyl-, (2Z)-	0.29
7.34	D-Glucose, 6-O-α-D-galactopyranosyl-	0.78
8.46	Ascaridole epoxide	0.03
8.71	4,9-Decadienoic acid, 2-nitro-, ethyl ester	0.07
9.35	Hexadecanoic acid, ethyl ester	0.05
9.59	10, 13-Octadecadiynoic acid, methyl ester	0.23
10.01	4-Decynoic acid, methyl ester	0.18
10.21	Caryophyllene	0.56
11.47	Benzoic acid, 3-hydroxy-	13.51
12.45	Ledol	1.19
13.89	Azulene, 1,4-dimethyl-7-(1-methylethyl)-	0.27
15.46	Ethanol, 2-(9-octadecenyloxy)-, (Z)-	0.18
16.10	Ethyl iso-allocholate	0.06
16.32	(7α-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	0.91
16.80	Aromadendrene oxide-(1)	0.75
17.25	n-Hexadecaonoic acid	0.44
17.70	2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	0.15
18.17	6,9,12,15-Docosatetraenoic acid, methyl ester	0.12
19.67	Phytol	3.18
19.95	Ethanol, 2-(9,12-octadecadienyloxy)-, (Z,Z)-	0.12
20.06	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	1.08
23.38	12-Bromo-13-hydroxy-2,5,9,13-tetramethyltetradeca-4,8-dienoic acid, methyl ester	2.52
25.11	Vitamin E	0.62

Table 3. Activity of phyto-components identified in *V. negundo* leaf extract by GC-MS.

RT	Name of the compound	Compound nature	Activity
6.17	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	Flavonoid fraction	Antimicrobial, antiinflammatory
7.34	D-Glucose, 6-O-α-D-galactopyranosyl-	Sugar moiety	Preservative
9.35	Hexadecanoic acid, ethyl ester	Palmitic acid ester	Antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
10.21	Caryophyllene	Sesquiterpene	Anti-tumor, analgesic, antibacterial, antiinflammatory, sedative, fungicide
11.47	Benzoic acid, 3-hydroxy-	Benzoic acid compound	Antimicrobial

Table 3. Contd.

12.45	Ledol	Sesquiterpene alcohol	Antimicrobial, antiinflammatory
16.10	Ethyl iso-allocholate	Steriod	
16.80	Aromadendrene oxide-(1)	Sesquiterpene oxide	Anti-tumor, analgesic, antibacterial, antiinflammatory, sedative, fungicide
17.25	n-Hexadecaonoic acid	Palmitic acid	Antioxidant, hypocholesterolemic nematicide, pesticide, anti androgenic flavor, hemolytic, 5-Alpha reductase inhibitor
18.17	6,9,12,15-Docosatetraenoic acid, methyl ester	Unsaturated fatty acid	Anti cholesterol compound
19.67	Phytol	Diterpene	Antimicrobial, anticancer, antiinflammatory, diuretic
20.06	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Linolenic acid	Antiinflammatory, hypocholesterolemic cancer preventive, hepatoprotective, nematicide, insectifuge, antihistaminic antieczemic, antiacne, 5-Alpha reductase inhibitor, antiandrogenic, antiarthritic, anticoronary, insectifuge
25.11	Vitamin E	Vitamin E	Antiageing, analgesic, antidiabatic antiinflammatory, antioxidant, antidermatitic, antileukemic, antitumor, anticancer, hepatoprotective, ypocholesterolemic, antiulcerogenic, vasodilator, antispasmodic, antibronchitic, anticoronary

**Source: Dr.Duke's phytochemical and ethnobotanical databases [Online database].

other activities.

ACKNOWLEDGEMENT

The authors thank very much Dr. K. Alagusundaram, Director, Indian Institute of Crop Processing Technology, Thanjavur for providing all the facilities and support used to carry out the work.

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