Oriental Journal of Chemistry Vol. **26**(4), 1501-1505 (2010)

DPPH Radical scavenging activity and antibacterial activity of *Pimenta dioica* (L.) Merr

B. HARI KUMAR[1], ANJUM BADARUDIN[1] and ANITHA JOSE[2]

[1]Department of Chemistry, T K M College of Arts and Science, Kollam - 691 005 (India).
[2]CEPC Laboratory, Kollam - 691 001 (India).

(Received: August 30, 2010; Accepted: October 11, 2010)

ABSTRACT

The essential oil of *Pimenta dioica* was isolated by hydro distillation and steam distillation. The aqueous extract was also taken. The antioxidant, total phenolic and the antimicrobial studies were conducted. The antimicrobial activity was significant. The IC_{50} value of extract and essential oil are 390 µg/ml and 2340 µg/ml respectively.

Key words: *Pimenta dioica*, DPPH, Antibacterial, Antifungal, Phenolic content, flavonoids, essential oil, aqueous extract.

INTRODUCTION

Pimenta dioica (L.)Merr. belongs to the botanical spice group of Pimenta Lindl., Myrtaceae family. The evergreen dried fruits and leaves *of Pimenta dioica* tree are used world wide as valuable spices. They are commonly known as allspice, clove pepper, English spice, Jamaican pepper, etc. Earlier the tree was grown mainly in Central America, Jamaica, Cuba, Brazil, etc, but now grown in India too.

The essential oils of *Pimenta dioica* leaves and fruits are utilized in food industry - mainly meat and canning industries as well as in perfumery compositions and cosmetic products. The therapeutic properties of the essential allspice oils are anaesthetic, analgesic, antimicrobial, antioxidant, antisceptic, carminative, muscle relaxant, rubefacient, stimulant and tonic. *Pimenta* oil can be helpful for the digestive system, for cramp, flatulence, indigestion and nausea. Further the essential oils can help in cases of depression, nervous exhaustion, tension, neuralgia and stress and is used as natural repellent. The essential oils of the leaves and fruits of this plant are also used in perfumes, aftershaves and commercial food flavouring.

The major compound of *Pimenta dioica* oil is eugenol (70-80%). In the *pimenta* leaf oils 1,8-cineole, α-humulene, β-caryophyllene and cadinene-derivatives were found as important constituents in higher concentrations[1,2].

Essential oils and extracts of *Pimenta* species are very interesting samples to investigate in this field, but only a few data are available until now [3-5]. The role of free radicals and reactive oxygen species in disease pathology is well established. Free radicals cause diseases like arteriosclerosis, arthritis, ischaemic heart attack, diabetis, neuro-degenerative diseases and others [6]. Currently there exists a great worldwide interest in finding new and safe antioxidants from natural resources.

MATERIAL AND METHODS

General

DPPH (1,1,-diphenyl-2-picrylhydrazyl) was

purchased from Sigma Aldrich. Gallic acid, Quercetin, BHA, BHT and α-tocopherol were purchased from Merck India, Mumbai. All other chemicals were of analytical reagent grade.

Plant material

The leaves of *Pimenta dioica* were collected from Kollam area of Kollam district in February 2010 and it was identified by Dr.N.Mohan, Scientist, TGBRI, Palode

Isolation of essential oil

The plant leaves were shade dried and cut into small pieces. The essential oils were obtained by two different methods. Dried samples (870 g) were homogenized and hydro distilled for six hours using a Clevenger type apparatus. To obtain essential oil by steam distillation (1200 g) material were subjected to steam for eight hours and then the volatile components in the steam were isolated by extraction with ether. All oils were subsequently dried over anhydrous sodium sulphate and stored in sealed vials until used. The essential oil isolated by steam distillation and hydro distillation from the sample gave 0.003437 g (w/w) and 0.003755 g (w/w) respectively. The essential oil obtained by hydro distillation was kept aside for comparing the chemical constitution.

Preparation of crude extract of *Pimenta dioica*

50 g of the dried plant material was grinded and then extracted with distilled water. The supernatent were evaporated and dried to get 18.3342 g (0.36668 w/w) of crude extract.

DPPH radical scavenging capacity

The DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging capacity was determined according to the method described by Braca.*et.al* [7]. Four test samples (1 ml, 2 ml, 3 ml and 4 ml) were taken and 1 ml of 0.008% methanol solution of DPPH were added and made up to 5 ml with alcohol or distilled water. A blank was also prepared. After 30 minutes absorbance was measured against the blank at 517 nm and the percentage inhibition activity was calculated as :

$$\text{Percentage inhibition activity} = 100 - (A_1/A_0) \times 100$$

where A_0 was the absorbance without sample and A_1 was the absorbance with sample. ∞-tocopherol, BHA, and BHT were used as standards (Table-1,2).

Estimation of total phenolic content

Total phenolic content (C) in the extract were estimated by the modified Folin Ciocalteu method [8,9]. An aliquot of the extract were mixed with 0.5 ml Folin Ciocalteu reagent and allowed to stand for 3 minutes followed by the addition of 2 ml of sodium carbonate. After one hour of standing the absorbance was measured at 630 nm. Total phenolic content was expressed as mg/g gallic acid equivalent and calculated as :

$$C = c.v/m$$

where C = total phenolic content in mg/g plant extract, c = concentration of gallic acid established via calibration curve, v = volume of plant extract, m = weight of plant extract (gm) (Table-3).

Estimation of total flavonoids

The method used for the estimation of total flavonoid content was modified by Jia.et.al. Quercetin was used as the standard. Five concentrations (1, 5, 10, 25 & 50 µg/ml) were prepared by dissolving quercetin standard in deionised water or ethanol. 2 ml from each concentrations were taken and labelled as S_1 to S_5. A blank was also prepared. 0.3 ml of 5% sodium nitrate solution was added and allowed to stand for 5 minutes followed by the addition of 0.6 ml 10% $AlCl_3$ solution. After 5 minutes of standing it was mixed 2 ml of 1M NaOH solution. The absorbance of the final solution was measured at 510 nm. Total flavonoids were expressed as mg/g quercetin equivalent and calculated as :

$$C = c.v/m$$

where C = total flavonoid content in mg/g plant extract, c = concentration of quercetin established via calibration curve, v = volume of extract, m = weight of plant extract (gm) (Table-3).

Antimicrobial activity

Five bacterial species representing gram positive and gram negative strains and fungii species for the testing purpose were obtained from MTCC, Institute of Microbial Technology, Chandigarh, India.

Microbial activity was evaluated by using a filter paper disc diffusion method. The degree of growth inhibition was evaluated after the incubation period of 24 hours at 37^{0}C. Three replicates of each were performed and the mean value was recorded.

RESULTS AND DISCUSSION

DPPH radical scavenging activity

The DPPH radical is widely used as a model to investigate the free radical scavenging of several plant extract. *Pimenta dioica* aqueous extract scavenged the DPPH radical in a concentration dependent manner. IC_{50} for the aqueous extract of *Pimenta dioica* was 390 µg/ml and for the essential oil is very high of 2340 µg/ml. The standards BHA, BHT and α-tocopherol showed IC_{50} = 840, 900, & 840 µg/ml respectively. This shows that the free radical scavenging activity of aqueous extract is much higher than the standard compound whereas the scavenging activity of essential oil is very less.

Total phenolic and flavonoid content

Polyphenolics are the major plant compounds with antioxidant activity and they play an important role in absorbing and neutralising free radicals, quenching singlets and triplet oxygen and

Table 1: Antiradical activity of aqueous extract of *Pimenta dioica* observed with DPPH

Samples	Concentration (µg/ml)	%Inhibition	IC_{50} (µg/ml)
Aqueous extract	100.84	10.37	390
	201.68	26.23	
	302.52	41.72	
	403.36	51.22	

Table 2: Antiradical activity of essential oil of *Pimenta dioica* observed with DPPH

Samples	Concentration (µg/ml)	%Inhibition	IC_{50} (µg/ml)
Essential oil	489.76	30.01	2340
	979.52	35.18	
	1469.28	35.50	
	1959.04	43.73	
BHA			840
BHT			900
α-tocopherol			840

Table 3: Total phenolic and flavanoid contents of *Pimenta dioica*

Sample	concentration (mg/g) equivalent
Aqueous extract	0.0524 mg/g gallic acid equivalent
Aqueous extract	0.0825 mg/g quercetin equivalent

Table 4: Antimicrobial activity of the essential oil of *Pimenta dioica*

Microorganisms	Growth inhibition (mm) due to essential oils and their different dilutions [a,b]									
	1:0	**1:1**	**1:2**	**1:4**	**1:6**	**1:8**	**1:10**	**1:12**	**1:14**	**1:16**
Bacteria										
Staphylococcus aureus MTTC -96	25	30	16	19	19	14	15	12	9	6
Escherichia coli MTTC -118	21	30	30	16	12	8	7	-	-	-
Salmonella typhi MTTC -733	22	21	17	16	15	11	11	7	-	-
Pseudomonas aeruginosa MTTC -424	24	30	20	18	18	14	11	8	6	-
Bacillus coreus MTTC -430	19	20	18	23	20	15	12	9	6	-
Fungii										
Saccharomyces cerevisiae MTTC -36	34						N D			
Aspergillus flavus MTTC -277	24						N D			
Candida utilis MTTC -183	12						N D			

[a]Including diameter of the filter paper disk; [b]Mean value of three independent experiments.
N D- Not determined

decomposing peroxides. The results from the study strongly suggest that the phenolics are important component of these plants and some of their pharmacological effects may be due to the presence of phenolics. The total phenolic content in mg/g gallic acid equivalent was found to be 0.0524 mg/g in aqueous extract of *Pimenta dioica*. The total flavonoids in mg/g quercetin equivalent were found to be 0.0825 mg/g in aqueous extract of *Pimenta dioica***.**

Antimicrobial

The essential oil of *Pimenta dioica* showed considerable microbiocidal activity. From the previous investigation it was suggested that the essential oil of *Pimenta dioica* can be used to develop potential herbal as well as biodegradable microbiocides[10]. The antibacterial and antifungal activities result seems to be inaccordance with previous results, indicating that the essential oil possess high levels of antimicrobial activity (Table 4). The antimicrobial activity clearly shows the inhibitory activity of the essential oil against microorganisms. The available literature shows that the essential oils are used in food technology[11], and pharmaceutical industry[12,13].

ACKNOWLEDGEMENTS

The author, Dr. Hari kumar. B thanks the University Grants Commission for the award of the minor project (MRP(S)-365/08-09/KLKE020/UGC-SWRO) and CEPC Laboratory, Kollam, for conducting the antimicrobial and antioxidant activity.

REFERENCES

1. Maringiu, B., Piras, A., Porcedda, S., Casu, R., Oierucci, P., *J. Essent. Oil Res.,* **17:** 530 (2005).
2. Tucker, A.O., Maciarello, J., Landrum, L.R.,

J. Essent. Oil Res., **3:** 195 (1991).
3. Kikuzaki, H., Hara, S., Kawai, Y., Nakatani, N., *Phytochemistry*., **52:** 1307 (1999).
4. Son, Y.K., Song, T.H., Woo, I.A., Ryu, H.S., *J. Food Sci. Nutr.,* **10:** 92 (2005).
5. Miyajima, Y., Kikuzaki, H., Hisamoto, M., Nakatani, N., *Biofactors.,* **21:** 30 (2004).
6. Lee, S.K., Mbwambo, Z.H., Chung, H., Luyengi, I., Gamez, E.J., Mehta, R.G., Kinghorn, A.D., Pezzuto, J.M., *Comb. Chem. High Throughput Screen.,* **1:** 35 (1998).
7. Braca, A., Tommesi, N.D., Bari, L.D., Pizza, C., Politi, M., & Morelli, I., *Journal of Natural Products.,* **64:** 892 (2001).
8. Wolf, K., Wux, Liu, R.H., *Journal of Agriculture and Food Chemistry*., **51:** 609 (2003).
9. Somes, J.R., Dins, T.C.P., Cuaba, A.P., and Ameida, L.M., *Free Rad*., **26:** 469 (1997).
10. J.E., Thoppil, M.J., Deena, K., Sreeranjini, *The International Journal Of Aromatherapy.,* **12:** 105 (2002).
11. Burt, S., *Int. J. Food Microbial*., **94 :** 223 (2004).
12. Sahin, F., Gullice, M., Dafeura, D., Sokmen, A., Sokmen, M., Poiesion, M., Agar, G., Ozer, H., *Food control*., **15:** 549 (2004).
13. Valero, M., Guina, M.J., *Int. J. Food Microbiol.,* **106:** 90 (2006).