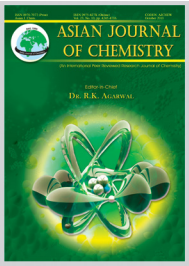
Asian Journal of Chemistry; Vol. 24, No. 7 (2012), 3243-3246

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in



Study of Chemical and Biological Aspects of *Valeriana wallichii* DC. Root Essential Oil

ZAHIDA PARVEEN*, SAIMA SIDDIQUE, MUAFIA SHAFIQUE, SHAISTA JABEEN KHAN and RAZIA KHANUM

PCSIR Laboratories Complex, Ferozpur Road, Lahore-54600, Pakistan

*Corresponding author: Fax: +92 42 99230705; Tel: +92 42 99230688-95 Ext: 318; E-mail: zahidaparveen18@gmail.com

(*Received*: 15 September 2011; *Accepted*: 24 February 2012) AJC-11113

The essential oil of *Valeriana wallichii* DC. root, which is an indigenous plant was extracted by hydro distillation. Chemical composition of the oil by GC-MS analysis revealed the presence of seven new components *i.e.* isoaromadendrene epoxide (3.435 %), isocaryophyllene (1.919 %), epiglobulol (1.692 %), longifolene-[V4] (1.618 %), panasinene (1.452 %), lognifolenaldehyde (0.875 %) and butanoic acid, 3-methyl-, 3-methylebutylster (0.840 %). Patchouli alcohol (16.425 %) was found to be the major component among ten identified components of the essential oil. Antibacterial activity and antioxidant activity of the said essential oil was investigated for the first time. The antibacterial potential of the essential oil of *V. wallichii* was determined by paper disc diffusion method, against Gram positive and Gram negative bacteria. Essential oil without any dilution was found to be more effective against all test microbes as compared to the standard antibiotics *i.e*. streptomycin and penicillin G. used as positive controls. DPPH scavenging effect was 96.47 % for essential oil of *V. wallichii*. Antioxidant activity of the oil was 11.27 % higher than BHT used as control.

Key Words: *Valeriana wallichii*, Valerianaceae, Essential oil, Gas chromatography, Antibacterial activity, Antioxidant activity, Patchouli alcohol.

INTRODUCTION

Valeriana wallichii DC (Asian specie) belongs to family Valerianaceae, has about 200 perennial herb and small shrub species throughout the world, is chiefly found in the temperate Himalaya and cold regions of the northern hemisphere. Other commonly used specie of genus valeriana include *V. officinalis* from Eastern Europe and *V. edulis* from Americas[1]. *V. wallichii* is an indigenous plant from Indian subcontinent used since long in Ayurvedic and Unani systems of medicine[2]. *Valeriana wallichii* DC is a small perennial herb of 14-45 cm height, with root stock, thick branching stem, sharply pointed leaves, white or pink flowers in clusters and hairy fruit. It occurs in Kashmir, Muree hills, Punjab and Northern areas of Pakistan[3,4]. Valerian is widely used as a mild sedative and sleep aid for insomnia, excitability and exhaustion. Other medicinal uses include diuretic, carminative antispasmodic, anticonstipation, anti-scorpion poison and to improve blood circulation[5].

Essential oils and extracts obtained from plants have recently gained a great popularity and scientific interest because of their relatively safe status, wide acceptance by consumers and exploitation for potential multi-purpose functional use[6]. Essential oils are concentrated, hydrophobic liquids containing volatile aroma compounds from plants. Various essential oils have been used medicinally at different periods in history. Valerian oil is obtained by steam distillation of the dried, ground roots and yield is reported to be between 0.2 and 0.7 %[1]. They are used in perfumes, cosmetics, soap and other products, for flavouring food and drink and for scenting incense and household cleaning products[5,7]. The antimicrobial and antioxidant activity of essential oils have great medicinal as well as food value.

Increase in the emergence of new bacterial strains that are multi-resistant, coupled with the non-availability and high cost of new generation antibiotics have resulted in increase morbidity and mortality[8]. Hence, there is need to look for potent antimicrobial and antioxidant agents from other sources. So, investigations of the antimicrobial activities, mode of action and potential uses of plant essential oils have regained momentum. Studies showed that most of the essential oils showed significant antimicrobial activity[7]. Literature indicates that studies on *V. wallichii* are scarcely found. Recently, essential oil of *V. wallichii* is reported to show the antifungal[9] and nematocidal activity[10]. However, its antibacterial and antioxidant activities are not yet reported.

The aim of present study is to determine the chemical composition, antimicrobial and antioxidant properties of essential oils from *V. wallichii* an indigenous Indian subcontinent plant specie.

EXPERIMENTAL

Extraction of essential oil: The *V. wallichii* roots were collected from the local market. They were cleaned from

extraneous matter. The essential oil was extracted through hydro-distillation by reverse Dean Stark assembly[11]. The steam distillate was removed, dried over anhydrous sodium sulphate and stored at 4 ºC for further studies.

GC-MS analysis: The analysis of the essential oil was carried out on GC-MS of Agilent Technologies, Model 6890N, operating in EI mode at 70 ev equipped with a split-splitless injector. Helium used as a carrier gas at the flow rate of 1 mL/min, while HP-5MS (30 m × 0.25 mm id, 0.25 µ film thickness) capillary column was used. The initial temperature was programmed at 50-140 ºC at the rate of 5 ºC/min and then 100-250 ºC at the rate of 3 ºC/min followed by a constant temperature at 260 ºC for period of 20 min. Sample (2 µL) was injected to column programmed at 200 ºC and resolutions of components were attained. The components were identified by their retention time and peak enhancement with standard samples in gas chromatographic mode and NIST library search from the derived fragmentation pattern of the various components of the oil.

Antibacterial assay: *In vitro* antimicrobial studies were carried out on eight bacterial strains including *Bacillus subtilis* ATCC6333, *Klebsiella pneumoniae, Salmonella typhimurium, Pseudomonas aeruginosa, Pseudomonas fluorescens, Staphylococcus aureus, Escherichia coli* and *Enterobacter aerogenes*. Among the tested microorganisms *Bacillus subtilis* ATCC6333 was obtained from microbiology laboratory of PCSIR labs complex Lahore and other were collected from pathological laboratory of a local hospital. All clinical isolates were characterized to specie level according to standard microbiological techniques described by Monica[12]. The cultures of bacteria were maintained in the laboratory on nutrient agar slants at 4 ºC throughout the study.

The paper disc diffusion method[13] was applied with slight modification to test the antimicrobial activity of *V. wallichii* essential oil. Normal strength nutrient agar medium (OXOID, England) was prepared and autoclaved at 121 ± 1 ºC for 15 min. For antibacterial assay 24 h old bacterial cultures grown at 37 ºC were used. Cultures were diluted 10^{-1} in sterile ringer solution[14] to set an inoculums density of approximately 10^6 CFU/mL, which was used for the test. 30 µL of these suspensions were inoculated to plates containing sterile nutrient agar medium using a sterile cotton swab.

Each filter paper discs (6 mm in diameter) impregnated with 25 µL of different concentrations of *V. wallichii* essential oil (pure oil, 1:1 and 1:5 dilution of oil in10 % aqueous solution of dimethyl sulfoxide) were placed on pre-inoculated culture media under aseptic conditions separately and incubated at 37 ºC for 24 h. The zone of inhibition was measured as the diameter (mm) of the clear zone around the discs. All experiments were performed in duplicate. PenicillinG and streptomycin were used as positive controls. Control antibiotics solution were prepared in appropriate amount (0.01 g/10 mL) then 25 µL of each antibiotic solution was dropped on paper discs and 10 % aqueous solution of dimethyl sulfoxide (DMSO) was used as negative control during present study.

Antioxidant activity

1,1-Diphenyl-2-picrylhydrazyl assay: 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test was performed to examine the antioxidant activity of *V. wallichii* oil. The scavenging effect on DPPH radical was determined by modifying the previous methods[15]. Briefly, 0.004 % DPPH solution was prepared in methanol. The essential oil was mixed with methanol in appropriate amounts to prepare 20, 40, 60, 80 and 100 % (v/v) methanolic test solutions. Butylated hydroxytoluene (BHT) was used as a positive control. A butylated hydroxytoluene stock solution (100 µg/mL) was used to prepare 20, 40, 60 and 80 %(v/v) methanolic solution, whereas butylated hydroxytoluene stock solution (100 µg/mL) was considered as 100 % working solution. Each methanolic dilution of essential oil as well as butylated hydroxytoluene (100 µL) was mixed with 3 mL of DPPH solution separately. The mixtures were shaken vigorously and left to stand for 0.5 h in dark at room temperature. The absorbance of the resulting solutions was measured at 517 nm using a UV-VIS spectrophotometer (Nicolet, Evlution-300, Germany). Decreasing absorbance of DPPH solution indicates an increase in DPPH radical scavenging activity corresponding to the antioxidant activity of the experimental *V. wallichii* essential oil. This activity is given as % inhibition, which is calculated with the following equation:

$$\text{DPPH scavenging effect (\% inhibition)} = \{(A_{blank} - A_{sample})/A_{blank}\} \times 100$$

where, A stands for absorbance. Each experiment was performed in triplicate.

RESULTS AND DISCUSSION

The essential oil was extracted by hydrodistillation from the *V. wallichii* roots, having 0.245 % (v/w) yield on dry weight basis. Present results indicate that the essential oil yield of *V. wallichii* roots (0.245 %) is higher than the claim made by Bos *et al.*[16] (0.09 %) on dry weight basis. The gas chromatography coupled with mass spectrometric analysis revealed the presence of 26 compounds, out of which 10 components were identified (Table-1). These components were further classified in two fractions *i.e*. hydrocarbon fraction and oxygenated fraction. Hydrocarbon fraction constituted camphene, isocaryophillene, isocaryophillene, panasinene while butanoic acid, 3-methyl-, 3-methylbutylester, bornyl acetate, epiglobulal, patchouli alcohol, isoarmadendrene epoxide, lognifolenaldehyde comprized the oxygenated fraction of the oil. Presence of patchouli alcohol (16.425 %) as major component of *V. wallichii* oil in present study is in accordance with the previous reports by Bos *et al.*[16] and Mathela *et al.*[17]. Formerly Nouye *et al.*[18] has reported the presence of bornyl acetate as a prominent component of *V. wallichii* oil, which is in accordance with the present results where bornyl acetate (2.546 %) is found to be third largest constituent of the oil. Camphene (1.522 %), butanoic acid, 3-methyl-, 3-methylebutylster (0.840 %), isocaryophillene (1.919 %), longifolene-[V4] (1.618 %), panasinene (1.452 %), epiglobulol (1.692 %) and lognifolenaldehyde (0.875 %) were present in considerable quantity.

Salient finding of the present investigation is the first time reporting for six newly identified components of the *V. wallichii* root essential oil including butanoic acid, 3-methyl-, 3-methylebutylester, isoaromadendrene epoxide, isocaryo-

TABLE 1
GS/MS ANALYSIS OF ESSENTIAL OIL OF *Valeriana wallichii* ROOTS

Name of compounds	Percentage (%)	m/e Values
Camphene	1.522	M^+136 (19 %), 121 (70 %), 107 (30 %), 93 (100 %), 79 (38 %), 67 (22 %), 53 (11 %)
Butanoic acid, 3-methyl-, 3-methylbutylester	0.840	M^+129 (6 %), 115 (6 %), 103 (21 %), 85 (67 %), 70 (100 %), 57 (42 %), 53 (3 %)
Bornyl acetate	2.546	M^+196 (4 %), 154 (14 %), 136 (52 %), 121 (52 %), 108 (22 %), 95 (100 %), 80 (15 %), 67 (14 %), 55 (14 %)
Isocaryophyllene	1.919	M^+204 (35 %), 189 (27 %), 161 (80 %), 133 (28 %), 119 (78 %), 105 (49 %), 93 (100 %), 79 (65 %), 55 (30 %)
Longifolene-[V4]	1.618	M^+222 (4 %), 204 (37 %), 189 (42 %), 161 (37 %), 133 (28 %), 119 (100 %), 105 (57 %), 77 (38 %), 69 (27 %), 55 (26 %)
Panasinene	1.452	M^+204 (21 %), 189 (9 %), 121 (99 %), 107 (13 %), 81 (53 %), 67 (29 %), 59 (100 %), 53 (1 %)
Epiglobulol	1.692	M^+222 (3 %), 204 (3 %), 189 (9 %), 149 (24 %), 126 (100 %), 121 (22 %), 108 (98 %), 81 (45 %), 67 (30 %) 55 (25 %)
Isoaromadendrene epoxide	3.435	M^+220 (100 %), 202 (23 %), 187 (30 %), 177 (93 %), 162 (39 %), 149 (67 %), 135 (35 %), 121 (50 %) 107 (59 %), 91 (55 %), 81 (55 %), 77 (31 %), 67 (25 %), 55 (25 %), 51 (5 %)
Patchouli alcohol	16.425	M^+222 (100 %), 207 (34 %), 189 (14 %), 179 (17 %), 161 (35 %), 138 (35 %), 121 (18 %), 109 (28 %), 98 (42 %), 67 (24 %), 55 (24 %), 51 (2 %)
Lognifolenaldehyde	0.875	M^+222 (73 %), 202 (39 %), 187 (45 %), 176 (55 %), 176 (55 %), 161 (56 %), 149 (22 %), 145 (27 %), 135 (43 %), 107 (100 %), 5 (81 %), 91 (71 %), 81 (66 %), 77 (44 %), 55 (49 %), 51 (5 %)

TABLE-2
ASSESSMENT OF ANTIMICROBIAL ACTIVITY OF *Valeriana wallichii* ESSENTIAL OIL AGAINST EIGHT SELECTED MICROBES

Test micro-organisms	Antimicrobial activity of *V. wallichii* essential oil and some standard Antibiotics. Zone of inhibition (mm)					
	Oil conc. pure 25 µL/D[a]	Oil conc. 1:1 25 µL/D	Oil conc. 1:5 25 µL/D	Streptomycin 25 µg/D	Penicillin G 25 µg/D	Dimethylsulfoxide 25 µL/D
Bacillus subtilis ATCC 6333	40.00 ± 0.31	32.00 ± 0.53	25.00 ± 0.26	36.00 ± 0.16	23.00 ± 0.70	-
Staphylococcus aureus HI[b]	38.00 ± 0.31	30.00 ± 1.02	23.00 ± 0.64	35.00 ± 0.25	22.00 ± 0.33	-
Salmonella typhimurium HI	37.25 ± 1.06	31.00 ± 1.41	22.25 ± 1.77	25.50 ± 0.71	-	-
Pseudomonas aeruginosa HI	31.25 ± 0.35	28.5 ± 0.70	22.75 ± 1.76	22.25 ± 1.06	-	-
Pseudomonas fluorescens HI	30.00 ± 0.21	26.00 ± 0.44	19.00 ± 0.36	22.00 ± 0.65	-	-
Klebsiella pneumoniae HI	34.00 ± 0.24	25.75 ± 1.06	16.50 ± 0.53	17.00 ± 0.01	-	-
Escherichia coli HI	35.50 ± 0.71	29.00 ± 0.35	21.50 ± 0.70	23.75 ± 0.35	-	-
Enterobacter aerogenes	35.75 ± 0.35	30.50 ± 0.70	25.00 ± 1.41	18.50 ± 1.12	-	-

[a]Paper disc (6 mm diameter); [b]Hospital isolated pathogen; ± Standard deviation; (-) No inhibition zone (resistant)

phillene, longifolene-[V4], panasinene, epiglobulol and lognifolenaldehyde.

The *in vitro* antimicrobial potential of *Valeriana wallichii* essential oil against Gram positive and Gram negative bacteria along with antibiotics as a positive control and DMSO as negative control is presented in Table-2. The results indicated that 25 µL of pure essential oil per disc showed significant antimicrobial activity against both Gram positive and Gram negative bacteria. Among Gram positive microorganisms under investigation pure essential oil of *V. wallichii* showed larger inhibition zone diameter (IZD) of 40.00 ± 0.31 mm against *B. subtilis* but the inhibition zone diameter was decreased to 32.00 ± 0.53 mm and 25.00 ± 0.26 mm as the dilution of the oil increased to1:1 and 1:5 respectively. The growth of *S. aureus* was restricted to inhibition zone diameter of 38.00 ± 0.31 mm, 30.00 ± 1.02 mm or 23.00 ± 0.64 mm for discs impregnated with pure or 1:1/1:5 dilutions of the essential oil respectively. In case of Gram negative bacteria six microbes namely *S. typhimurium, P. aeruginosa, P. fluorescens, K. pneumoniae, E. coli* and *E. aerogenes* were investigated. The results indicated that pure essential oil showed superior inhibition zone diameter (37.25 ± 1.06 mm) against *S. typhimurium* followed by 31.00 ± 1.41 mm and 22.25 ± 1.77 mm at 1:1 and 1:5 dilutions of the essential oil respectively. *E. aerogenes* was found to be the next sensitive bacteria having inhibition zone diameter of 35.75 ± 0.35, 30.50 ± 0.70 and 25.00 ± 1.41 mm against pure essential oil, 1:1 and 1:5 dilutions of the essential oil respectively. Subsequently, inhibition zone diameter of 35.50 ± 0.71, 29.00 ± 0.35 and 21.50 ± 0.70 mm was noted for the above mentioned experimental concentrations of the essential oil in case of *E. coli*. On the other hand *K. pneumoniae* was found to be less sensitive as compared to *S. typhimurium, E. aerogenes* and *E. coli*. against all three concentrations of *V. wallichii* essential oil producing inhibition zone diameter of 34.00 ± 0.24, 25.75 ± 1.06 and 16.50 ± 0.53 mm along the descending order of the essential oil concentration. In case of *P. aeruginosa* inhibition zone diameter *i.e*. 31.25 ± 0.35, 28.5 ± 0.70 and 22.75 ± 1.76 mm at three experimental concentrations of essential oil were noted. *P. fluorescens* was found to the least sensitive organism

showing the smallest inhibition zone diameters *i.e.* 30.00 ± 0.21, 26.00 ± 0.44 and 19.00 ± 0.36 mm for pure essential oil followed by at 1:1 and 1:5 dilutions of the essential oil.

All the plants differ significantly in their activities against the micro-organisms. Current finding supports the fact that *V. wallichii* essential oils are effective to check the growth of certain Gram positive and Gram negative bacteria. The oil exhibited great antimicrobial activity and produced inhibition zones with the diameters ranging from 40.00 ± 0.31 to 16.50 ± 0.53, which is higher than streptomycin in certain cases used as positive control (Table-2). Nematocidal activity of *V. wallichii* oil is already reported by Kim *et al.*[10] whereas antifungal potential of methanolic crude extract of *V. wallichii* has been explored for the first time by Shalini[9]. An antibacterial activity profile is reported in this study for the first regarding essential oil of *V. wallichii* cultivars found in Pakistan.

There is an extensive evidence to implicate free radicals produced in the biological redox reactions which leads to the development of degenerative diseases such as diabetes, liver cirrhosis, nephrotoxicity, cancer *etc.*[19]. Natural compounds show strong antioxidant activity which is likely to quench radicals[20]. Reducing power is an indicator of the antioxidant activity of a given reagent/product. The ability of essential oil to act as a donor for hydrogen atom or electrons in the transformation of DPPH radical into its reduced form DPPH-H measuring the antioxidant activity of the entity under investigation. The results of DPPH scavenging activity of *V. wallichii* root oil compared with butylated hydroxytoluene as a positive control are shown in Fig. 1. These results indicate that *V. wallichii* root oil has higher antioxidant activity as compared to butylated hydroxytoluene at all concentration levels *i.e.* 20-100 %. Antioxidant activity of essential oil (100 %) was recorded to be 11.27 % higher than the corresponding level of butylated hydroxytoluene as shown in Fig.1. No reports about the antioxidant potential of *Valeriana wallichii* are so far found in the literature. This is the first time report for antioxidant activity of *V. wallichii* essential oil.

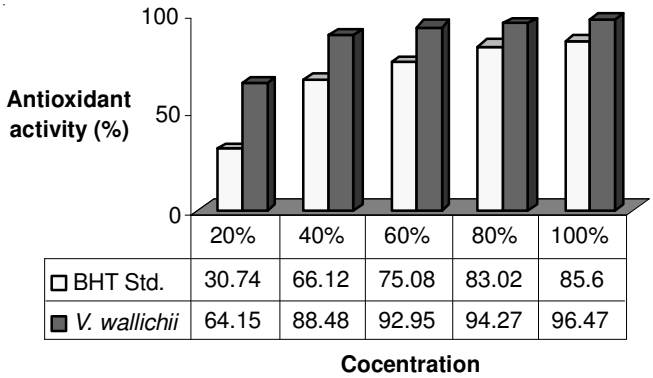


Fig. 1. Percentage antioxidant activity of essential oil of *V. wallichii* DC. root in comparison with BHT by DPHH assay

Conclusion

In conclusion, this the first study evaluating the antibacterial and antioxidant activities of essential oil of *V. wallichii*. The results has shown that oil has strong *in vitro* antibacterial avtivity against *B. subtilis* ATCC 6333, *K. pneumoniae, S. typhimurium, P. aeruginosa, P. fluorescens, S. aureus, E. coli* and Enterobacter and antioxidant activity. So, the essential oil can be used in treatment of diseases caused by these microbes directly or by incorporation into medicines used for the treatment of these ailments. Further studies are required to determine the mechanism of action of the essential oil for antibacterial and antioxidant activity.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Zia-ur-Rehman, Senior Scientific Officer, PCSIR Laboratories Complex, Lahore, Pakistan for GC-MS analysis.

REFERENCES

1. M.K. Rangahau, *Crop. Food Res*. [Broad sheet], 34 (2001).
2. S. Sati, C.S. Chanotiya and C.S. Mathela, *J. Essent. Oil Res*., **17**, 408 (2005).
3. S.R. Baquar, Medicinal and Poisonous Plants of Pakistan, Printas, Karachi, p. 479 (1989).
4. K.M. Nadkarni, Indian Medica, Popular Prakashan, Bombay, p. 1260 (1976).
5. T.O. Odugbemi, Outlines and Pictures of Medicinal Plants from Nigeria, University of Lagos Press, Lagos, Nigeria, p. 91 (2006).
6. X. Ormancey, S. Sisalli and P. Coutriere, *Perfumes, Cosmetiques, Actualites*, **157**, 30 (2001).
7. G. Sacchetti, S. Maietti, M. Muzzoli, M. Scaglianti, S. Manfredini, M. Radice and R. Burni, *Food Chem.*, **91**, 621 (2005).
8. K. Lewis and F. Ausubel, *Nature Biotech.*, **24**, 1453 (2006).
9. S.R. Shalini, *Elec. J. Environ. Agric. Food Chem.*, **8**, 218 (2009).
10. J. Kim, S.M. Seo, S.G. Lee, S.C. Shin and I.K. Park, *Agric. Food Chem.*, **56**, 7316 (2008).
11. A. Sattar, Proceeding of the First National Symposium on Essential Oil, Perfumes and Flavour, PCSIR Laboratories Complex, Lahore, pp. 7-12(1989).
12. M. Cheesbrough, District Laboratory Practice in Tropical Countries, Cambridge University Press, Part II, p. 63 (2000).
13. A.W. Bauer, M.D.K. Kirby, J.C. Sherris and M. Turck, *Am. J. Clin. Pathol.*, **45**, 493 (1966).
14. C.H. Collins, Microbiological Methods, London, Butterworths, p. 335 (1967).
15. W. Brand-Williams, M.E. Cuvelier and C. Berset, *Lebensm.-Wiss Tech.*, **28**, 25 (1995).
16. R. Bos, H. Woerdenbag, H. Hendriks, H.F. Smith, H.V. Wikstrom and J.J.C. Scheffer, *Flav. Fragrance J.*, **12**, 123 (1997).
17. C.S. Mathela, M. Tiwari, S.S. Sammal and C.S. Chanotiya, *J. Essent. Oil Res.*, **17**, 672 (2005).
18. S. Nouye A. Amin, F. Karim, M. Amano and S. Abe, *Aroma Res.*, **8**, 149 (2007).
19. S.N. Laxane, S.K. Swarnkar and M.M. Setty, *Pharmacologyonline*, **1**, 319 (2008).
20. K. Youdim, H.D. Dorman and S.G. Deans, *J. Essent. Oil Res.*, **11**, 643 (1999).