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Chemical Composition and Antimicrobial Activity of Essential Oil of *Viburnum betulifolium*

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The composition of the hydrodistilled essential oil of *Viburnum betulifolium* (Caprifoliaceae) was analyzed by GC and GC-MS. Fourty six compounds were identified representing 90.8% of the total oil. Phytol (9.8%), Trans-b-Damascenone(5.9%), α-Cadinol(5.7%) , γ-Cadinene (5.6%), Δ-Cadinene (5.3%), Methyl pentanoate (4.6%), Tetradecanal (3.8%), Pentadecanal (3.3%) α- Calcorene (2.4%), 2-Pentylfuran (2%) and 4-Terpineol (1.9%) were characterized as the main constituents. The essential oil was tested for its antimicrobial activity using a micro-dilution assay resulting in the inhibition (MIC: 62.5-250 μg mL^{-1}) of human pathogenic bacteria and yeast. However, the essential oil of the *V. betulifolium* showed strong antimicrobial activity against Gram (+) and Gram (-) human pathogenic bacteria and the yeast *Candida albicans*. This is the first report of the essential oil constituents of *V. betulifolium* and antimicrobial activity.

Key words: *Viburnum betulifolium*, essential oil, antimicrobial activity, ampicillin, fluconazole

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INTRODUCTION

Essential oil and their constituents are widely exploited in the traditional medicine and their curative potentials are well documented (Dubey *et al*., 2004). Infectious diseases are the leading cause of death worldwide. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. Natural products, as a pure compound or as a standardized plant extracts and essential oils provide unlimited opportunities for new drug lead because of the unmatched availability of chemical diversity. An increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic and infectious agents has led to the screening of medicinal plants for their antimicrobial potential. In recent studies have shown that natural products and especially essential oils and components thereof display potential as antimicrobial agents for various uses in medical applications (Parekh and Chanda, 2007; Hammer *et al*., 1999; Dorman and Deans, 2000). The genus *Viburnum*, belonging to the Caprifoliaceae family, consist of about 230 species distributed in subtropical and warm temperate regions, 80 of which are distributed in china. *V. betulifolium,* ever green shrub with green leaves and white flowers is widely distributed throughout the Yunnan Province and southwestern part of China (Wang *et al*., 2008). *Viburnum* species are widely used in folk medicine for the treatment of Antispasmodic, diuretic, sedative properties and uterine excitability (Cometa *et al*., 1998). Previous phytochemical studies on *Viburnum* species have shown the presence of different natural compounds including iridoids, coumarins, triterpenoids, vibsane-type diterpenes, phenolic glycosides and lignans (Kagawa *et al*., 1998, Hase *et al*., 1985, Fukuyama *et al*., 1996, Zhu *et al*., 2006). The essential oil studies on some *Viburnum* species also reported (Nagihan *et al*., 2008). However, to the best of our knowledge, there is no previous study on the essential oil of *V. betulifolium*. The only previous study on this species reported the occurrence of triterpenoid (Hu *et al*., 2011). Therefore, the objective of the present study was to examine the chemical composition of the essential oil of *V. betulifolium* by Gas Chromatography-Mass Spectrometry as well as to evaluate the antimicrobial activities of the essential oils.

MATERIALS AND METHODS

Plant material: The leaf part of *V. betulifolium* was collected in July 2008 from Caojian town in Yunnan Province in China. The sample was identified by Prof. Xiao Cheng and a voucher specimen has been deposited in the Kunming Institute of Botany, Chinese Academy of Sciences, China.

Isolation of essential oil: Fresh leaf parts of the plant material (300 g) were subjected to hydro-distillation for 5 h using a Clevenger-type apparatus to yield the essential oil (1.35 mL).Essential oil yield was 0.45%.The essential oil collected was dried over anhydrous sodium sulphate and stored at 4°C until the analysis was carried out.

Gas chromatography: The essential oil was analysed by GC using a Thermo Fisher TRACE GC ULTRA coupled with DSQ II Mass Spectrometer instrument using a TR 50MS column (60 m×0.25 mm inner diameter, with 0.25 μm, film thickness) was used with helium as carrier gas (1 mL min^{-1}). The oven temperature was kept at 70°C for 10 min and programmed to 220°C at rate of 3°C/min, then kept constant at 220°C for 10 min and then programmed to 240°C at rate of 1°C/min. The injector temperature was 250°C. Relative percentages (i.e., percent peak area relative to total peak area) were obtained from electronic integration measurement using a flame ionization detector (FID, 250°C), n-Alkanes were used as reference point in the calculation of Retention Indices (RI).

Gas chromatography-mass spectrometry: The sample was analyzed by GC/MS employing the same chromatographic conditions as described above, using a Thermo Fisher TRACE GC ULTRA coupled with DSQ II Mass Spectrometer instrument with helium as carrier gas (1 mL min^{-1}). Split ratio was adjusted at 50:1, MS were recorded at 70 eV. Mass range was from 50 to 650 m/z, Library search was carried out using the (NIST/Wiley) and confirmed by comparison of their retention indices with data published in literature (Kagawa *et al*., 1998).

Antimicrobial assay: The micro-dilution broth susceptibility assay was used for antimicrobial evaluation of the essential oil (Koneman *et al*., 1997). A stock solution was prepared in dimethyl sulphoxide (DMSO) and the dilution series were prepared up to 0.97 μg mL^{-1} using sterile distilled water in 96-well micro-liter plates.

Over-night grown microbial suspension in double strength Mueller Hinton broth and yeast suspension of *Candida albicans* in yeast medium were standardized to approximately 10^8 CFU mL^{-1}

(using McFarland No: 0.5), 100 μL of each well. The last row containing only the serial dilutions of antimicrobial agents without microorganism was used as negative control. Sterile distilled water and medium served as a positive growth control. After incubation at 37°C for 24 h the first well without turbidity was determined as the minimal inhibition concentration (MIC).Human pathogens were obtained both from the culture collection of the Microbiology Department in King George's Medical University Lucknow, India. Ampicillin was used as standard antibacterial agent for bacteria. Whereas Fluconazole was used for *C. albicans.*

RESULTS AND DISCUSSION

Hydrodistillation of leaf part of *V. betulifolium* afforded colorless pleasant smelling oil. The constituents of oil identified indicated in Table 1. A total of 46 compounds, resulting 90.8% of the total essential oil. Phytol (9.8%), trans-b-Damascenone (5.9%), α-Cadinol (5.7%), γ-Cadinene (5.6%), Δ-Cadinene (5.3%), Methyl pentanoate (4.6%) and Tetradecanal (3.8%) were the main constituents of the essential oil of *V. betulifolium.* The antimicrobial activity of the essential oil against Gram (+) and Gram (-) human pathogenic bacteria and the yeast *Candida albicans.* A micro-broth dilution assay was used to determine the Minimum Inhibitory Concentration (MIC) of the essential oil against five different microorgamisms.It showed good inhibitory activity against the Gram (-) human pathogen *P. aerugenosa* (MIC 125 μg mL^{-1}) and the yeast *C. albicans* (MIC 62.5 μg mL^{-1}). Some inhibition against the other tested bacteria was observed when compared to the standard agent ampicillin. The detail results of inhibitory concentrations against the used microorganisms can be seen at Table 2.

The antibacterial activity of the volatile oils tested was more pronounced against Gram-positive than against Gram-negative bacteria. This generally higher resistance among Gram-negative bacteria could be ascribed to the presence of their outer membrane, surrounding the cell wall, which restricts diffusion of hydrophobic compounds through its lipopolysaccharide covering (Ratledge and Wilkinson, 1988). The absence of this barrier in Gram-positive bacteria allows the direct contact of the essential oil's hydrophobic constituents with the phospholipids bilayer of the cell membrane, causing either an increase of ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems (Wendakoon and Sakaguchi, 1995; Cowan,1999).

Table 1: Composition of the essential oil of *V. betulifolium*

Compounds	RI	Oil (%)	Method of identification
Methyl pentanoate	827	4.6	RI, MS
(Z)-3-Hexen-1-ol	856	1.7	RI, MS
n-Heptanal	902	1.9	RI, MS
2-Pentylfuran	994	2.0	RI, MS
Phenyl acetaldehyde	1043	1.9	RI, MS
Linalool oxide	1076	1.6	RI, MS
Terpinolene	1085	0.5	RI, MS
Linalool	1095	1.4	RI, MS,CI
n-Nonanal	1101	0.9	RI, MS
4-Terpineol	1176	1.9	RI, MS,CI
α-Terpineol	1186	1.7	RI, MS,CI
Methyl salicylate	1191	1.8	RI, MS,CI
Myrtenol	1195	0.9	RI, MS
n-Decenal	1203	0.8	RI, MS
trans- Carveol	1220	0.6	RI, MS
Geraniol	1256	1.7	RI, MS,CI
2E-Decenal	1265	1.8	RI, MS
Cinnamaldehyde	1272	tr	RI, MS
2E,4Z-Decadienal	1291	1.7	RI, MS
2E,4E-Decadienal	1316	1.8	RI, MS
α-Copaene	1375	0.7	RI, MS
trans-β-Damascenone	1383	5.9	RI, MS
trans-α-Ambrinol	1416	1.3	RI, MS
α-Amorphene	1484	1.2	RI, MS
Germacrene D	1486	1.1	RI, MS
β-Ionone	1488	1.9	RI, MS
α-Muurolene	1504	tr	RI, MS
γ-Cadinene	1516	5.6	RI, MS
Δ-Cadinene	1524	5.3	RI, MS
trans-Cadina-*1,4*-diene	1536	1.8	RI, MS
α- Calcorene	1544	2.4	RI, MS
Ledol	1572	0.8	RI, MS
Spathulenol	1581	tr	RI, MS
Caryophyllene oxid	1585	tr	RI, MS
Salvial-4(14)-en-1-one	1596	tr	RI, MS
Tetradecanal	1613	3.8	RI, MS
α- Muurolol	1644	3.6	RI, MS
β- Eudesmol	1652	tr	RI, MS
α- Cadinol	1655	5.7	RI, MS
Occidenol	1676	tr	RI, MS
Pentadecanal	1716	3.3	RI, MS
Manool	2058	0.9	RI, MS
n- Heneicosane	2101	1.9	RI, MS
Phytol	2115	9.8	RI, MS
n-Docosane	2203	1.2	RI, MS
n-Tricosane	2301	1.4	RI, MS

RI: Retention indices, MS: Mass spectrum, CI: Co-injection with authentic standards, Tr: Trace (<0.1%)

Table 2: Antimicrobial activity of *V. betulifolium* essential oil (MIC in μg mL^{-1})

Microorganisms	Source	EO	St[a]
Staphylococcus aureus	ATCC 6538	125.0	15.62[a]
Escherichia coli	ATCC 25792	125.0	62.5[a]
Bacillus subtilis	ATCC 6633	250.0	31.25[a]
Pseudomonas aeruginosa	ATCC 27853	125.0	250.00[a]
Candida albicans	ATCC 10231	62.5	125.00[b]

EO: *V. betulifolium* essential oil, ST: Standard agent [a]Ampicillin, [b]Fluconazole

CONCLUSION

In present study, the essential oil of *V. betulifolium* was tested for its antimicrobial activity against human pathogenic bacteria and yeast *C. albicans.* It showed good inhibitory activity against the Gram (-) human pathogen *P. aerugenosa* and the yeast *C. albicans* .Some inhibition against the other tested bacteria was observed when compared to the standard agent ampicillin. The study has proved that essential oil of *V. betulifolium* show significant antimicrobial activity.

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