Original Article

Major Constituents, Antioxidant and Antibacterial Activities of Zanthoxylum armatum DC. Essential Oil

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Abstract

Chemical compositions, antioxidant and antibacterial activities of Zanthoxylum armatum essential oil was analyzed. A total of 3 samples (500 g/each) were collected at 6:00 am; 12:00 noon; and 6:00 pm in the same day. The essential oil was extracted by hydro distillation in Clevenger apparatus and their chemical compositions were determined by the GC-MS system. The eleven most abundant ingredients were bornyl acetate (16.61-22.66%), cymene (8.25-12.50%), α-copaene (7.54-7.59%), γ-terpinene (5.33-7.66%), camphene (4.32-4.66%), limonene (2.66-4.68%), linalool (3.28-3.58%), β-ocimene (3.24-3.36%), trans-caryophyllene (2.54-3.46%), α-terpinolene (2.32-3.36%) and germacrene (2.02-2.85%). Antioxidant activity was examined by 2, 2’-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. Ascorbic acid was used as standard. Essential oil exhibited significant antioxidant activity. The IC₅₀ of the oil was found 27.0± 0.1 μg/ml and that of ascorbic acid 15.0± 0.5 μg/ml. Essential oil of Z. armatum also exhibited moderate antibacterial activity. The results showed that the gram-positive bacteria are more sensitive to the essential oil than gram negative bacteria.

Keywords: Zanthoxylum armatum, Essential oil, antioxidant, anti-bacterial, GC-MS, DPPH, Ciprofloxacinc

Zanthoxylum are deciduous aromatic shrubs and trees (family Rutaceae) comprises 250 species native to warm temperate and subtropical region of the world. Traditionally, leaves and fruits are used for mouth fresh and tooth care while bark is used for intoxicating the fishes [1] and leaves, fruits and barks are also used as spice [2]. Plants essential oils, commonly used as fragrances and flavoring agents for foods and beverages, are also recommended as an alternative source for constituting numerous bioactive phytochemicals that can be potentially used for insect control [3]. The main components of Zanthoxylum oil are oleic acid, palmitic acid, linoleic acid methyl ester, limonene and linalool [4]. β-Caryophyllene, α- and β-farnesene, β-bisabolol, γ-cadinene, nerolidol, 2-undecanone and dodecanal were identified from the essential oil of Z. gillettii [5]. Chemical constituents of Z. bungeanum and Z. piperitum essential oils have been determined [6,7]. Altitudinal variations of linalool and limonene in leaves of Z. alatum have also been determined by Gupta et al. [8].

Medicinal plants are good source of remedies for human health problems due to the presence of bioactive compounds. Recently wide ranges of medicinal plants have been screened for antioxidant and antimicrobial activities [9-11]. These medicinal plants are also used in different countries as a source of many potent and powerful drugs. Antimicrobial property of essential oils of medicinal plants have been reviewed [12]. Zanthoxylum tingoassuiba and Z. hyemale essential oils have also been reported for their antimicrobial activity [13,14] and Z. leprieurii and Z. xanthoxyloides for their antioxidant activity [15]. In view of strong antioxidant and antimicrobial properties of essential oils, the present study was designed to determine major constituents, antioxidant and antibacterial activities of Zanthoxylum armatum essential oil.
Table 1. Chemical composition of essential oil of Z. armatum

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 am</td>
</tr>
<tr>
<td>Camphene</td>
<td>4.59</td>
</tr>
<tr>
<td>γ-Terpinene</td>
<td>5.38</td>
</tr>
<tr>
<td>Cymene</td>
<td>8.25</td>
</tr>
<tr>
<td>Limonene</td>
<td>3.26</td>
</tr>
<tr>
<td>β-Ocimene</td>
<td>3.24</td>
</tr>
<tr>
<td>α-Terpinolene</td>
<td>2.32</td>
</tr>
<tr>
<td>Linalool</td>
<td>3.28</td>
</tr>
<tr>
<td>Bornyl Acetate</td>
<td>17.82</td>
</tr>
<tr>
<td>Trans-Caryophyllene</td>
<td>3.46</td>
</tr>
<tr>
<td>Germaacrene</td>
<td>2.02</td>
</tr>
<tr>
<td>α-Copaene</td>
<td>7.54</td>
</tr>
</tbody>
</table>

MATERIALS AND METHODS

Plant material and extraction of essential oil

Leaves of Z. armatum were collected from Mandal forest of Uttarakhand, India. A total of 3 samples (500 g/each) were collected at 6:00 am; 12:00 noon; and 6:00 pm in a same day. The plant was authenticated through existing literature (herbarium No. GUH 3802). The fresh leaves of Z. armatum were cut into small pieces and subjected to hydro-distillation in a Clevenger apparatus (5 h). The pale yellow colored essential oils were collected, dried over anhydrous sodium sulphate and stored in a sealed glass vials at low temperature (0-4°C) prior to analysis.

Gas chromatography-mass spectrometry (GC-MS) analysis

Qualitative and quantitative analysis of extracted essential oils of Z. armatum were performed on Perkin-Elmer make Clarus-500 GC equipped with Perkin-Elmer-Clarus-500 MS and capillary column (60m×0.25mm, film thickness 0.25 µm). Injector and detector temperatures were 210°C and 280°C, respectively, while the helium was used as carrier gas. Oven temperature was held for 5 minutes at 50°C with 5 min solvent delay, then programmed at 3°C/min up to 220°C/min, and then maintained isothermally at 220°C for 20 min. GC-MS was operated in EI mode at 70 eV.

Antioxidant activity

DDPH radical scavenging activity of Z. armatum oil isolated from the sample collected at 6 pm (high yield) was determined by well diffusion method described according to Cutler and Wilson [18] and Gupta et al. [19] with slight modifications. Bacterial cultures of Staphylococcus faecalis, Staphylococcus aureus, Proteus vulgaris and Klebsiella pneumoniae were obtained from Department of Microbiology, HNB Garhwal University and used for antibacterial test organism. The bacteria were maintained on nutrient broth at 37°C. The gram positive (Streptococcus faecalis and Staphylococcus aureus) and gram negative (Proteus vulgaris and Klebsiella pneumoniae) bacteria were precultured in nutrient broth overnight in incubator. The stock culture suspensions were diluted with sterile Saline water. The Petri dishes were flooded with Mueller Hinton Agar and after solidification of agar 0.1 ml of diluted inoculums were spread over Mueller Hinton Agar in the dishes using sterile L spreader to achieve confluent growth. Six millimeter diameter wells were cut from the agar using a sterile cork borer, and 10 µl of the essential oil was delivered into the wells. Ciprofloxacin (5 µg/well) was used as standard. The antibacterial assay plates were incubated at 37°C for 24 h. The diameters of the zone of inhibition (ZoI) were measured in mm.
Table 2. Comparative accounts of major constituents (%) of Zanthoxylum oil

<table>
<thead>
<tr>
<th>Species</th>
<th>Plant part</th>
<th>γ-Terpinene</th>
<th>Limonene</th>
<th>β-Ocimene</th>
<th>α-Terpinolene</th>
<th>Linalool</th>
<th>Bornyl Acetate</th>
<th>Trans-Caryophyllene</th>
<th>Germacrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z. armatum</td>
<td>Seeds[21]</td>
<td>-</td>
<td>19.8</td>
<td>-</td>
<td>-</td>
<td>57.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Leaves[8]</td>
<td>-</td>
<td>1.59-2.76</td>
<td>-</td>
<td>-</td>
<td>34.06-35.57</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Z. bungeanum</td>
<td>Fruits[22]</td>
<td>7.30</td>
<td>-</td>
<td>1.10</td>
<td>-</td>
<td>3.70</td>
<td>0.30</td>
<td>0.60</td>
<td>-</td>
</tr>
<tr>
<td>Z. acanthopodium</td>
<td>Leaves[23]</td>
<td>0.40</td>
<td>14.80-18.30</td>
<td>0.30</td>
<td>0.80</td>
<td>0.10</td>
<td>2.2-2.6</td>
<td>0.6</td>
<td>-</td>
</tr>
<tr>
<td>Z. rhoifolium</td>
<td>Leaves[39]</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>34.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Fruits[29]</td>
<td>-</td>
<td>31.09</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Z. limonella</td>
<td>Fruits[30]</td>
<td>6.60</td>
<td>12.90</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Leaves[30]</td>
<td>-</td>
<td>33.10</td>
<td>6.20</td>
<td>-</td>
<td>23.30</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Z. schinifolium</td>
<td>Fruits[38]</td>
<td>-</td>
<td>14.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Zanthoxylum species</td>
<td>Leaves[27]</td>
<td>-</td>
<td>12.00</td>
<td>-</td>
<td>-</td>
<td>13.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Chemical composition of essential oil

The hydro-distillation of Z. armatum yielded pale yellow colored oil (yielded–0.088%-0.176%, w/v). The individual constituents separated by gas chromatography were identified by comparing their MS with those of standard NIST (National Institute of Standards and Technology, U.S. Department of Commerce) and Wiley (John Wiley & Sons Ltd) libraries. Upon GC-MS analysis, the hydro-distilled oils were found to contain forty two constituents eluted between 18 and 65 min. Among detected constituents eleven are found to be major constituents, which are mainly comprised of mono and sesquiterpenoids (Table 1). Comparative accounts of major constituents of Zanthoxylum oils are summarized in Table 2. The lowest yield (0.088%) was recorded for the sample harvested at 12:00 noon, and the highest (0.176%) was obtained at 6:00 pm. Thus it can be perceived that to obtain the highest essential oils yield, the collection must be comprehended near about 6:00 pm. Variation in the aerial part and seed essential oil yield of the genus Zanthoxylum were observed 0.12-0.42% [20] and 1.3-1.36%, respectively. In many other reports [21,22] 0.019% and 0.017% dry weight essential oil was also recorded in Z. acanthopodium leaves collected in summer and winter, respectively [23]. Quantitative differences can be observed, when a comparison is made between the different daytime collections.

The major constituents were found in the range 2.02-22.66%. Among these, bornyl acetate was the major component (16.61-22.66%) followed by cymene (8.25-12.50%), α-copaene (7.54-7.59%), γ-terpinene (5.33-7.66%), limonene (2.66-4.68%), camphene (4.32-4.66%), linalool (3.28-3.58%), β-ocimene (3.24-3.36%), trans-caryophyllene (2.54-3.46%), α-terpinolene (2.32-3.36%) and germacrene (2.02-2.85%). Bornyl acetate was identified as the main constituent, which was found with the concentration of 22.66% at 6:00 pm, 17.82% at 6:00 am and 16.61% at 12 noon. And germacrene was present at the lowest concentration of 2.02% at 6:00 am, 2.53% at 12 noon and 2.85% at 6:00 pm. The mean values of % composition of three collections are presented in Table 1. Bisht and Chanotiya [24] analyzed the essential oil of Z. armatum leaf by capillary gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) and found 2-undecanone as main component followed by 2-tridecanone. Although linalool and limonene were also reported as dominant content in several other studies on Z. armatum oil [21,25]. There are number of reports [26,27] on the essential oil analysis of Zanthoxylum. One study showed that linalyl acetate, linalool and limonene were major components in the essential oil of Zanthoxylum [26]. GC-FID and GC-MS analysis of the essential oil of Z. schinifolium and Z. bungeanum showed that limonene was the main component, followed by 4-terpineol, γ-terpine, α-terpineol acetate, β-pinene, α-terpineol and β-linalool [28]. Ithiphanichpong et al. [29] had also reported limonene, terpin-4-ol and sabine as major components of essential oil of Z. limonella Alston. Another study showed that major components in the fruit oil were linalool, limonene, aterpineol, α-pinene, γ-terpine, cis-β-octimene, terpinen-4-ol and isomenthone, while in leaf oil, the predominant was limonene, followed by geraniol, carvone, 7-hydroxy-3,7dimethyloctanil, geranial and nerol [30]. Our results
The activity of Z. chalybeum S. aureus was the most sensitive (20 mm) while the gram presence of exhibited moderate (23 mm) and lowest against E. coli Proteus vulgaris positive bacteria, Rui Zanthoxylum 1997; Gaur RD. Flora of the District Garhwal North West Himalaya: to the, 29 essential oil could be associated with its main and bornyl acetate followed by cymene, α bornyl trans for ascorbic acid was 15.0± 0.5 μg/ml. Shigella boydii Z. armatum - and 10 This investigation reveals that essential oil of Zhong Yao Cai i 38 reported for their activities were quite high content of Staphylo Pestic antifungal activity. The study revealed that essential oil of Z. armatum essential oil may be due to the difference in their antibacterial activity. Their antibacterial activity of Z. armatum is an excellent antioxidant and also exhibited moderate antibacterial activity. Its activities were quite comparable with the standards. Antioxidant and antibacterial activities of Z. armatum essential oil may be due to the presence of terpenoids. The study also reveals that the consumption of Zanthoxylum would exert several beneficial effects by virtue of their antioxidant and antibacterial activities.

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**References**


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