



CHEMICAL CONSTITUENTS, ANTIMICROBIAL POTENTIAL AND ANTIOXIDANT EFFICACY OF ESSENTIAL OIL FROM *BOESENBERGIA PULCHERRIMA* (WALL.) KUNTZE

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Abstract

Infectious microorganisms and oxidative stress are cause of severe disease and are still a major threat to public health. A tremendous effort has been made in controlling the widespread of the same. In this context, several plants and their products are naturally used as antimicrobial and antioxidant agents. Wild species from the Zingiberaceae family meet these demands to a large extent. To evaluate the behavior of essential oil from *Boesenbergia pulcherrima* was extracted by hydrodistillation technique. The essential oil was characterized by GC-MS profiling, unveiled the occurrence of 21 major volatile constituents. Palmitic acid (75.56%) was found to be most predominant compound followed by spathulenol (7.51%), palmitoleic acid (5.51%), humulene epoxide II (3.06%) and citronellyl formate (3.05%). Antimicrobial activity indicates *Shigella flexneri* and *Aspergillus flavus* was highly susceptible to *Boesenbergia pulcherrima* essential oil when compared to *Klebsiella pneumonia* and *Aspergillus parasiticus*. *In vitro* antioxidant activity was evaluated using 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Reducing Power Assay and Total Antioxidant Capacity which depicts a promising antioxidant activity when compared to the standard Ascorbic acid. Hence, *Boesenbergia pulcherrima* essential oil comprised potential chemical constituents which could be commercially exploited in several fields including food, pharmaceutical, nutraceutical and cosmetic industries.

Key words: Antimicrobial activity, antioxidant efficacy, *Boesenbergia pulcherrima*, essential oil, volatile constituents.

Introduction

Essential oils (EO) are natural, volatile and complex aromatic compounds characterized by strong odour and found as potential secondary metabolites in plants. Generally, they obtained by steam or hydrodistillation process, developed firstly by the Arabs during the times of Middle Ages. Essential oils known for their medicinal properties such as antiseptic, bactericidal, fungicidal, virucidal and they are used in embalmment and preservation of foods also they were used in antimicrobial, sedative, analgesic, anti-inflammatory, spasmolytic and local anaesthetic remedies. Many essential oils are extracted from higher plants and possess antimicrobial activity against various pathogenic microorganisms (Singh and Upadhyay, 1993; Lis-Balchin *et al.*, 1996). In the traditional system of Indian medicine, spices are major contributor to aromatics and are antiseptic, stomachic, carminative, stimulants and prevent flatulence (Chopra, 1956; Singh, 2002).

Infectious microorganisms cause a severe disease and are still a major threat to public health despite tremendous efforts has been made in controlling the widespread of infections. Though a great number of antibiotics are now available, many of the infections exhibit resistance. In addition, studies indicate that antibiotics have multiple side effects in some

individuals such as hypersensitivity (Rosato *et al.*, 2007). Further studies indicate that synergetic effect of essential oil along with antibiotic could be best possible way to rule out some of the side effects and to increase the antimicrobial activity (Rodrigues *et al.*, 2009; van Vuuren and Viljoen, 2011; Duarte *et al.*, 2012), as majority of antibiotic compounds are either plant or bacterial sources. Hence, the research community has been trying to isolate newer drugs for controlling some of the infections arising out of pathogenic microorganisms that are devoid of adverse side effects.

Oxidative stress is a major cause of several severe diseases including cardiovascular and neurodegenerative diseases (Dhalla *et al.*, 2000; Chen *et al.*, 2012). Several antioxidants have been isolated naturally to effectively fight against reactive oxygen species. In this context, several plants and their products are naturally used as antioxidants. Wild spices have been found to be rich source of antioxidants including Zingiberaceae family. *Boesenbergia pulcherrima* is a wild aromatic plant belonging to Zingiberaceae family and endemic to Western Ghats of Southern India. It is reported across the Western Ghats region of Karnataka and Kerala growing in evergreen forests (Sabu, 2006) genus *Boesenbergia* comprises 80 species (Aishwarya *et al.*, 2015).

Several studies have reported that the *Boesenbergia* spp. have been used as spices in several South-East countries like Thailand (Trisonthi and Trisonthi, 2006) and Singapore (Skornickova and Gallic, 2010) for their numerous medicinal properties such as antibacterial (Hwang *et al.*, 2004; Zainin *et al.*, 2013), antifungal (Jantan *et al.*, 2003; Phongpaichit *et al.*, 2005), antioxidant (Shindo *et al.*, 2006), anti-inflammatory (Tewtrakul *et al.*, 2009), antiulcer (Abdelwahaba *et al.*, 2011), antiviral (Cheenpracha *et al.*, 2006), antitumor (Murakami *et al.*, 1993) and anticancer (Win *et al.*, 2007). Further, several novel bioactive compounds have been reported from this genera, especially *Boesenbergia rotunda* is the most extensive studied plant wherein more than hundred compounds have been isolated so far (Eng-Chong *et al.*, 2012). However, nutritional analysis of the *B. pulcherrima* has been reported elsewhere, information on volatile constituents is not reported so far, hence in the present study, an investigation was carried out to determine chemical composition, antimicrobial and antioxidant potential of *B. pulcherrima* essential oil.

Materials and Methods

Plant Material

Boesenbergia pulcherrima was collected from the Agumbe (Longitude 13°29'51.3"N and Latitude 75°04'60.0"E), in September 2016. A voucher specimen is deposited at Department of Botany, Karnatak University, Dharwad, India. Further, the plant was authenticated by Prof. Sabu, Department of Botany, Calicut University, Kerala, India.

Microorganisms

The microorganisms were procured from microbial type culture collection centre, IMTECH-Chandigarh, India. Bacterial cultures used in this study are: *Staphylococcus epidermidis* MTCC 435; *Escherichia coli* MTCC 40; *Shigella flexneri* MTCC 1457; *Enterobacter aerogenes* MTCC 2822; *Proteus mirabilis* MTCC 425; *Staphylococcus aureus* MTCC 6908; *Enterococcus faecalis* MTCC 6845; *Proteus vulgaris* MTCC 744 and *Klebsiella pneumoniae* MTCC 9238. Fungal strains are *Aspergillus parasiticus* MTCC 2796; *Aspergillus flavus* MTCC 8790; *Aspergillus fumigatus* MTCC 8877.

Essential Oil Extraction

The essential oil was extracted from the whole plant (leaves, rhizomes) by hydrodistillation using Clevenger type of apparatus for 4 hours, according to the procedure of the European Pharmacopoeia 4.

Gas Chromatography-mass Spectrometry (GC-MS) Analysis

The essential oil composition was determined by gas chromatography-mass spectrometry technique using Shimadzu GCMS-QP2010S model. GC-MS is fitted with a fused silica ZB-5 (5% phenyl methyl siloxane) capillary column (30 m × 0.25 mm, 0.25 µm film thickness). Column temperature was programmed from 60°C to 240°C at 3°C/min, and helium was used as carrier gas (1.1 mL/min). Ionization of the sample components was performed in the EI mode, (70 eV), with scan range 40-450 amu and 1 µl of essential oil was directly injected.

Antimicrobial Tests

In vitro antimicrobial activity of essential oil of *B. pulcherrima* was evaluated by the disc diffusion method. All tests were performed in Mueller-Hinton agar, dimethyl sulfoxide (DMSO, 3.2%) was used as solvent for making the various concentrations of essential oil (EO). Each microorganism was suspended in sterile saline (0.9%) solution and diluted to 100 CFU/mL. 0.45-µm sterile discs were thoroughly moistened with 20µL of different concentrations of oil. The standard antibiotic (streptomycin) was also tested against all pathogens. The antibacterial activity of essential oil and antibiotic was demonstrated by a clear zone of inhibition around the disc. The zone of inhibition was measured after 24-hour incubation at 37°C. The experiment was performed in duplicates.

In vitro Antioxidant Activity

DPPH Radical Scavenging Activity

The DPPH activity was carried out for the EO following the protocol followed by Blois in (1958) with certain modification, as follows. Briefly 0.3mM of DPPH solution prepared in methanol and was mixed to various concentrations of EO sample (200-1000 µg/mL) and standard Ascorbic acid. Essential oil and Ascorbic acid were prepared in methanol. To the test solution (2.5 mL), DPPH solution (0.3 mM, 1 mL) was added, vortexed and incubated in dark for 30 minutes at 37±1°C. Blank was prepared without EO samples, absorbance was recorded at 517nm. Percentage inhibition activity was calculated by following formula: % inhibition = $(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}} \times 100$, where, A=absorbance at 517 nm.

Reducing Power Activity

Reducing power assay or Phosphomolybdenum assay of EO was determined by the following method as described by Oyaizu *et al.* (1986). In brief, plant essential oil of various concentrations 200-1000 µg/ml was taken in a series of test tubes, final volume is made up to 1.0 ml of methanol, to which 2.5 ml of 0.2 M

phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide was added and incubated at 50°C for 20 min in water bath. Further 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 650 rpm for 10 min. 2.5 ml of supernatant was taken out and mixed with 2.5 ml distilled water to which finally 0.5 ml of FeCl₃ (0.1%) was added. Absorbance was recorded at 700 nm against blank using a double beam UV-Visible spectrophotometer. Increased absorbance of reaction mixture shows increased reducing power.

Total Antioxidant Capacity

The total antioxidant activity of the plant EO was determined by following protocol of Prieto *et al.* (1999) with certain modifications in quantity. 0.2 ml of essential oil of various concentrations (200, 400, 600, 1000 µg/ml) was taken in a test tubes to which 1.8 ml of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was added. The tubes were then incubated at 95°C for 90 min in a water bath and allowed to cool. The absorbance was measured at 695 nm against the blank using double beam UV-Visible spectrophotometer. The values are represented as µg ascorbic acid equivalents per gram dry weight.

Results and Discussion

Physical observation of oil was found to be pale yellow in colour and gave a distinctive strong aroma. GC-MS analysis reveals the presence of 21 essential oil constituents. Essential constituents of the oil are tabulated in table 1. Palmitic acid (75.56%) was found to be most predominant compound followed by spathulenol (7.51%), palmitoleic acid (5.51%), humulene epoxide II (3.06%) and citronellyl formate (3.05%). In the present study it was observed that the Palmitic acid was major constituent of oil which has been also reported in plants such as *Acer nigrum* essential oil comprising 39.1 % to 77.2 %. Further, plants such as *Allium jesdianum* Boiss, *Allium ursinum* L., *Prunella vulgaris* L. (70.0 %), *Lycium chinense* (62.89 %) *Scutellaria diffusa* (30 %) have been also reported to constitute rich content of palmitic acid (hexadecanoic acid) (Chen *et al.*, 2012; Amiri, 2007; Blazewicz-Wozniak and Michowska, 2011; Chung *et al.*, 2011; Cicek *et al.*, 2011; Rouis-Soussi *et al.*, 2014). A recent study showed that *Etilingera elatior* Jack., essential oil comprised 27% of hexadecanoic acid (Wijekoon *et al.*, 2013). However, the earlier report suggests that hexadecanoic acid (Palmitic acid) is most common among the spices and exhibits properties such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and cardio-protective (Suhaj, 2006). Thus, components of essential oil are therapeutic in nature and exhibit potential bioactivities.

Spathulenol is one of the significant components rectified and is also sesquiterpene which are basically identified in the essential oils of medicinal plants. Spathulenol is abundantly found in *K. sicula* and many other plant species (Rang *et al.*, 2004; Djarri *et al.*, 2008; Mendes *et al.*, 2008). Those plant species have proved to be significant factor in anticancer, antimicrobial and immunomodulator effects, whereas, Spathulenol is consider as a base module in diverse sectors such as foods, medicines, toothpaste, detergents and cosmetics (Mendes *et al.*, 2008; Martins *et al.*, 2010; Ziaei *et al.*, 2011). In spite of that various extracts of plant species were utilized in traditional medicine system for long time. Basically they are used to treat in antimicrobial disease which helps to increase microorganism resistance to antibiotic drugs. The ethanol extracts from *Centratherum punctatum*, the aerial parts possess cytotoxic potentials which also include the existence of anticancer compounds; spathelenol by using Trypan blue dye exclusion method (Sivasubramanian and Brindha, 2013).

Palmitoleic acid is the third major compound in *B. pulcherrima* essential oil, earlier it was reported by Cao *et al.* (2008) and Erbay *et al.* (2009), that animal models involved for metabolic disease, where the adipose tissue releases the monounsaturated fatty acid, palmitoleic acid [16:1n-7 (cis-9-hexadecenoic acid)], which help in decreasing hepatic steatosis and improves insulin sensitivity in the whole body. Fatty acid promising phenomena for anti-inflammatory lipid which may help to ameliorate metabolic disorders (Cao *et al.*, 2008; Erbay *et al.*, 2009). Recent studies revealed that monounsaturated fatty acids are beneficial to human diet (Schwingshackl and Hoffmann, 2012). The proportion of saturated to unsaturated fatty acids comprises a significant property of the phospholipid component of biological membranes. Variation in this proportion are thought to exhibit deleterious effects on cells and, in particular, decrease in the number of unsaturated fatty acids in membranes usually contributes to the growth of a number of pathophysiological states, such as cardiovascular disease, diabetes and cancer (Schwingshackl and Hoffmann, 2012). Accordingly, monounsaturated fatty acids are humorous to biological membranes due to liquid at body temperature, which are not easily oxidized, they constantly help in retaining membrane fluidity within the appropriate limits.

Humelene epoxide II is the fourth major component which has been reported by Nyiligira *et al.* (2004) as the significant essential oil component, due to presence of Humelene epoxide II it helps in certain activities such as antimicrobial and anti-inflammatory activities. Citronellyl formate is also one of the major components responsible for biological activity. The

therapeutic activity of the oil proved to be efficient in treating for dysentery, diarrhoea, biliary conditions, gastric ulcers, diabetes, cancer and skin diseases (Monika *et al.*, 2012).

Antimicrobial activity was evaluated against different pathogens that included nine bacterial species and three fungal species (Table 2). Streptomycin was taken as standard reference antibiotic. All the microorganisms used in the present study were opportunistic and infectious. Among bacteria *Klebsiella pneumonia* was found to be resistant against both the essential oil and streptomycin. Whereas in fungi *Aspergillus fumigatus* was resistant. However, in bacteria *Shigella flexneri* was found to be more susceptible to essential oil with 17.1 ± 1.0 mm zone of inhibition, followed by *Escherichia coli* (16.5 ± 0.7) *Staphylococcus epidermidis* 14.5 ± 0.2 mm zone of inhibition. *Aspergillus flavus* was more susceptible with 13.2 ± 0.7 mm zone of inhibition among fungal strains. Several authors have recorded the antimicrobial efficacy of *Boesenbergia* spp. against numerous pathogens including food borne pathogens (Bhamarapravati *et al.*, 2006; Voravuthikunchai *et al.*, 2006; Jing *et al.*, 2010), the results clearly denoted that *B. pulcherrima* essential oil was antagonistic to most of the tested pathogens and could be readily used in treatment of infections. This plant could find immense applications in the pharmaceutical and food industries.

Antioxidant activity was evaluated with the aid of three *in vitro* assays. Results revealed that essential oil was able to scavenge free radicals in dose dependent manner (Fig. 2 and 3). Fig. 2 explains the antioxidant potential of essential oil and standard ascorbic acid against DPPH radical. It could be observed that 10mg/ml exhibited 95% of free radical scavenging

activity. Total antioxidant activity revealed that essential oil comprised a total of 1000 $\mu\text{g/ml}$ of ascorbic acid equivalent antioxidant capacity. Reducing power assay (Fig. 3) indicated that with increased concentration of essential oil increased absorbance was noted. Thus all the three assays provided preliminary evidence against essential oil's antioxidant potential. The antioxidant nature of essential oil can be attributed to the aggregated effect of various chemical constituents present. Abdelwahab *et al.* (2011) confirmed the antioxidant activity of *Boesenbergia rotunda* rhizome methanolic extract using two assays viz., DPPH and FRAP assay. Further, Isa *et al.* (2012) reported that boesenbergin A, a chalcone derivative isolated from *B. rotunda* rhizome exhibited antioxidant activity.

Conclusion

In conclusion numerous studies have shown that essential oil could be beneficial against several diseases. In the present study it was observed that the Palmitic acid (hexadecanoic acid) was major constituent of oil, this volatile compound is therapeutic in nature and exhibits potential bioactivities such as antioxidant, antimicrobial, anti-inflammatory, hepatoprotective and cardio-protective. In the present study an investigation was made to evaluate the chemical constituents of *Boesenbergia pulcherrima* and evaluation of its antimicrobial and antioxidant potential. The results revealed that essential oil of *Boesenbergia pulcherrima* was effective against tested pathogens and was able to scavenge free radicals in a dose dependent manner against standard ascorbic acid. Thus, the plant can be exploited for the use in food, pharmaceutical and nutraceutical industries. However, detailed studies involving *in vivo* models are quite essential to confirm its therapeutic nature.

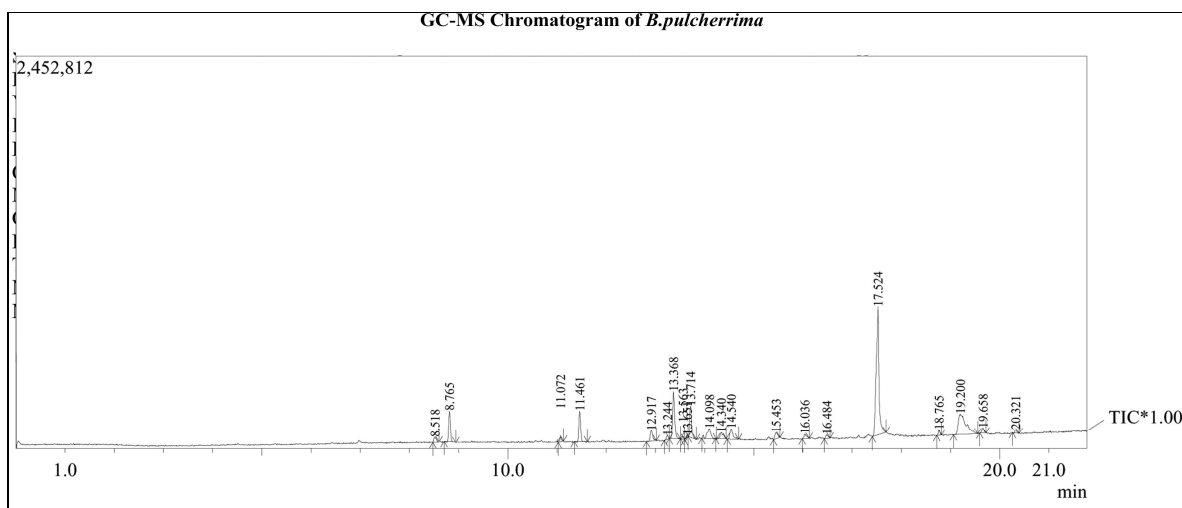


Fig. 1 : GC-MS Chromatogram of *B. pulcherrima* essential oil.

Table 1 : Constituents of essential oil of *B. pulcherrima*.

Peak	RT (min)*	Compounds**	Area (%)
1	8.518	Geraniol	0.109
2	8.765	Citronellyl formate	3.049
3	11.072	1,8-cineole	0.058
4	11.461	Bornyl formate	2.631
5	12.917	Nerolidol	0.475
6	13.244	Menthol	0.042
7	13.368	Spathulenol	7.512
8	13.563	α -terpineol	0.018
9	13.651	Dodecanal	0.008
10	13.714	Humulene epoxide II	3.055
11	14.098	Humulene epoxide III	0.694
12	14.340	(E)-linalool oxide	0.187
13	14.540	(Z,E)-farnesol	0.559
14	15.453	Pentadecanoic acid	0.180
15	16.036	Pentadecanal	0.085
16	16.484	Nonadecanal	0.058
17	17.524	Palmitic acid	75.56
18	18.765	Tetradecanal	0.077
19	19.200	Palmitoleic acid	5.514
20	19.658	Hexadecanal	0.090
21	20.321	Eicosanol	0.029

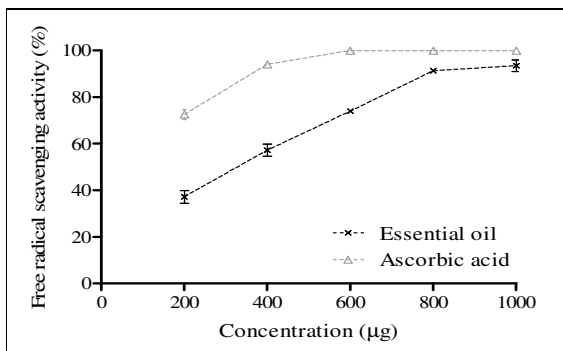
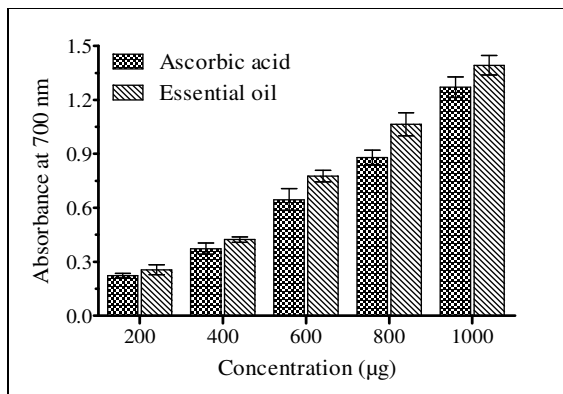
*Retention Time (RT)

**Tentative assignments of compounds based on GC-MS results.

Table 2 : Zone of Inhibition exhibited by *B. pulcherrima* essential oil against infectious microorganisms.

Microorganisms (MTCC accession number)	<i>B. pulcherrima</i> essential oil (mm)	Antibiotic Streptomycin (mm)
Bacteria		
<i>Staphylococcus epidermidis</i> MTCC-435	14.5 \pm 0.2	24.5 \pm 1.3
<i>Escherichia coli</i> MTCC-40	16.5 \pm 0.7	24.4 \pm 1.3
<i>Shigella flexneri</i> MTCC-1457	17.1 \pm 1.0	28.2 \pm 0.9
<i>Enterobacteria erogenes</i> MTCC-2822	14.3 \pm 0.6	25.6 \pm 1.2
<i>Proteus mirabilis</i> MTCC-425	13.2 \pm 0.4	24.5 \pm 2.2
<i>Staphylococcus aureus</i> MTCC-6908	12.2 \pm 0.4	29.2 \pm 0.4
<i>Enterococcus faecalis</i> MTCC-6845	11.5 \pm 0.2	17.3 \pm 1.4
<i>Proteus vulgaris</i> MTCC-744	12.6 \pm 0.3	24.8 \pm 1.6
<i>Klebsiella pneumonia</i> MTCC-9238	-	-

Fungi		
<i>Aspergillus parasiticus</i> MTCC-2796	-	-
<i>Aspergillus flavus</i> MTCC-8790	13.2 \pm 0.7	16.2 \pm 1.9
<i>Aspergillus fumigatus</i> MTCC-8877	11.6 \pm 0.3	24.2 \pm 2.4

**Fig. 2 :** DPPH activity of *B. pulcherrima* essential oil.**Fig. 3 :** Reducing power assay of *B. pulcherrima* essential oil.

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