Essential Oil Composition, Antimicrobial Potential, Phytochemical Profile and Toxicity of Essential Oils and Crude Extracts of Sweet Basil Prior and After Flowering

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Abstract: Essential oils and crude extracts of sweet basil (*Ocimum tenuiflorum* - OT) which are mostly used to flavor and spice food were prepared and analyzed prior and after flowering by GC and subjected to *in vitro* antimicrobial and *in vivo* toxicity evaluation. The essential oil composition was found to vary considerably with respect to harvesting time (prior to flowering stage- eugenol- 17.7%, carvacrol- 27.1% and methyl-eugenol- 52.1%; as compared to flowering (caryophyllene- 9.26% and methyl-eugenol and comparatively smaller percentages of carvacrol and eugenol). Phytochemical screening of the plant samples from either season showed that the whole plants constituted of alkaloids, flavones, saponins, terpenes, sterols, tannins and triterpenes. The essential oils and crude extracts of plant parts of OT (leaves, twigs and/or inflorescence) in both stages showed marked antimicrobial activity against *S. aureus* and methicillin-resistant *S. aureus* and with no apparent toxicity in rats. The observed antimicrobial properties and non-toxic nature during both stages of flowering would tend to validate the medicinal properties of OT as a functional food.

Keywords: *Ocimum tenuiflorum,* essential oil, antimicrobial, toxicity, flowering, functional food, phytochemicals.

INTRODUCTION

Several species of sweet basil - *Ocimum tenuiflorum* (OT, syn. *O. sanctum*), have been reported as an effective functional food in African and Asian traditional recipes. Scientific evidences are available on various medicinal aspects i.e. antimicrobial, adaptogenic, antidiabetic, hepato-protective, anti-inflammatory, anti-carcinogenic, radioprotective, immunomodulatory, neuro-protective, cardio-protective and mosquito repellent amongst others. The essential oils and extracts of several species of the *Ocimum* genus from various geographical origins have been investigated for their composition and chemotaxonomy by many researchers. Several species of this genus have also been reported as effective drugs for many applications in African and Asian traditional medicine [1-2]. In Mauritius the two most famous *Ocimum* species are *Ocimum basilicum,* sweet basil and *Ocimum tenuiflorum* L. (syn *Ocimum sanctum* L.), commonly known as Tulsi. These two *Ocimum* species are among the most important representative of the 60 *Ocimum* species, and numerous varieties, belonging to the lamiaceae family. Locally, sweet basil is mostly used to flavor and spice food and the leaf infusion has also been reported as being very effective against mild upper respiratory infections, bronchoplasm and stress-related skin disorder. The leaves in the form of paste are applied on cutaneous lesions and ring worm. Infusion of the seeds is used to treat bronchial asthma and essential oil of the leaves is locally used to cure ear infections. These remedies most probably had their roots in the Indian Ayurveda and hence the knowledge inherited from the Indian immigrants that came to Mauritius [2-3]. The aims of the present study were to assess the biological activity of the local cultivar of sweet basil and geared towards determining (1) the major constituent of the essential oil obtained prior to and after flowering, (2) the antimicrobial potential of the essential oils as well as crude extracts of leaves, twigs and flower at both stages, and (3) to evaluate any possible toxicity of these extracts *in vivo* in an endeavour to validate its use as a functional food.

MATERIALS AND METHODS

Plant Material

Ocimum tenuiflorum L- Lamiaceae plants were collected prior (late July) and after flowering (late February), in the area of Ecroignard, the north-eastern part of the island where the plant grows wildly. Identification was confirmed by a local botanist.

Extraction of Essential Oils

For essential oil extraction, the fresh samples where thoroughly washed, cut to pieces and used for steam

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ISSN: 1927-3037/12

distillation. The essential oils of *Ocimum tenuiflorum* collected prior and after flowering were both obtained by hydro distillation of the freshly cut samples, using a Clevenger apparatus for 4 hrs. The distillates thus acquired were extracted with hexane (3 x 100 ml) and after drying with anhydrous sodium sulphate, the organic layer was evaporated under vacuum. The essential oils were collected in brown vial, stored in the refrigerator and percentage yields recorded.

Preparation of Crude Extracts

The crude extracts were prepared as described previously [4-5]. Briefly, dried plants were separated into leaves, stems and flowers (when in the flowering stage). Each part were grounded separately and stored in a dry and dark place. The methanolic extracts were prepared by maceration of the grounded plant materials (20 g) in methanol (200 ml) for a period of 24 hrs, after which, it was filtered, the methanol evaporated to dryness under vacuum and the mass of crude extracts, after lyophilization, were recorded. The same procedure was followed for the preparation of crude aqueous extracts, only, maceration were done in distilled water (200 ml). All extracts were labeled and stored in the refrigerator for antimicrobial and phytochemical screening.

GC-FID Analysis

GC/FID analysis was carried out using a GC-14A with SPME sleeve adapted to an injector, FID and C-R6A-Chromatopac integrator (Shimadzu, Japan). The carrier gas was nitrogen, the flow rate 0.9 ml/ min, the injector temperature 250 °C, the detector temperature also 250 °C and the column, a CP-SIL 5 CB (dimethyl siloxane) type of inner diameter of 0.32 mm. At the injection point, the apparatus waits for 0.5 min before it becomes ready for the analyte to be injected. The temperature program was: 60 °C for 0.5 min, then temperature was increased to 200 °C (rate of 8 °C/min), followed by a plateau for 2 min before increasing to 250 °C (rate of 10 °C/min), this temperature was maintained for 1 min and finally was allowed to decrease back to 60 °C (rate of 30 °C/min). Identification was achieved *via* the internal standard method, external standard method and correlations between retention times (Kovats retention index) and quantifications were done using peak area calculations [6-7].

Antimicrobial Assay

Agar dilution tests were performed according to standard methods with minor modifications [4]. Quality control microorganisms included *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Candida albicans*, *Aspergillus niger* and clinical isolated strains obtained from the Victoria Hospital namely *Salmonella typhi* ATCC 14028 and methicillin-resistant *S. aureus* (MRSA) ATCC 43300. The lowest concentration of extract or essential oil showing no growth was read as the Minimum Inhibitory Concentration (MIC). Positive control used was ampicillin and amphotericin B [4].

Toxicity Assay

Male Swiss albino rats (mean weight 100±50 g) were used for the *in vivo* acute toxicity tests as described previously [8] and as per international guidelines for testing of chemicals *in vivo*. All the animals were acclimated in cages under standard environmental conditions of light and dark cycles and maintained at room temperature and had free access to tap water and standard pellet diet. Daily cumulative oral administration of test samples was administered *via* a catheter over 14 days; the first concentration done according to the proportion 80 mg/Kg body weight. The animals were divided into 5 batches, the control and four treated groups, each consisting of 4 rats. Batch 1 was used to analyze the toxicity of aqueous leaf extract of *O. tenuiflorum* prior to flowering, batch 2 for the aqueous leaf extract after flowering, batch 3 and 4 for essential oils extracted before and after flowering season respectively. The crude plant extracts were weighed accordingly, dissolved in distilled water (0.25) and orally administered *via* a soft piece of rubber tubing to the specimens. The essential oils were homogenised in tween 80 and distilled water before injection. All the animals were subjected to 4 hours of fasting prior to treatment. They were observed for one hour after treatment, and then intermittently for four hours and during the 14 days following treatment. Clinical signs such as weakness, aggressiveness, loss of weight, diarrhoea, discharge from eyes and ears and the number of deaths in each batch were closely monitored as described previously [7-8].

Phytochemical Screening

Leaves, twigs and flowers were subjected to a thorough phytochemicals screening using standard protocols [5] to detect the presence of the following secondary metabolites: alkaloids, coumarins, terpenes, anthraquinones, tannins, phenols, leucoanthocyanins, flavones, and saponins. TLC (5 µl of a 100 mg

extract/ml solution) was on Silica Gel 60 coated on glass plates (Merck TLC F254) with hexane/ethyl acetate 1/1 (v/v) and DCM/methanol/water 65/35/0.5 (v/v/v) as eluants. The separated components were visualised under visible and ultraviolet light (254 and 360 nm, Camag Universal UV lamp TL-600) or using spray reagents such as 5% anisaldehyde in a 5% sulphuric acid in ethanol solution, vanillin and Dragendorff [3-5].

RESULTS AND DISCUSSION

The amount of essential oil of OT varied from one stage to the other. Irrespective of flowering season, essential oils were of a bright yellow colour and had a characteristic spicy odour with mild camphor note and with an undertone of clove. The physicochemical properties of the essential oils were markedly different, which from the start hinted at different compositions (relative density; 0.971 g/ml, 1.175 g/ml and refractive index; 1.53, 1.49 recorded for essential oil prior and after flowering respectively). The major constituents of the essential oil extracted before flowering were mainly eugenol (17.7%), carvacrol (27.1%) and methyl-eugenol (52.1%) while for the essential oil extracted after flowering, was chiefly δ-cadiene (21.1%), caryophyllene (14.2%), methyl-eugenol (50.1%) and traces of carvacrol and eugenol (< 3%). Indeed, Ramesh *et al*. [9] have assessed the volatile constituents in essential oil from different plant parts of methyl-eugenol rich OT and has showed that inflorescences had higher oil content as compared to leaves. However, the leaf oil consisted of higher amount of methyl-eugenol which indicated their equal significance in total methyl-eugenol production of the present chemotype assessed. Interestingly, the higher yield of oil from samples collected after flowering in the present study, confirmed earlier observation, in that, inflorescence contributed more oil than leaf. Nonetheless, the relatively low change, or decrease, in methyl-eugenol content from the samples after flowering as compared to the oil obtained before flowering, suggests that the inflorescence of the local OT contribution of methyl-eugenol was negligible. The actual chemical composition of essential oil is a function of species, chemotype, climate, soil conditions and geographical location; it is thus not surprising to come across varied major constituents reported in the literature on of essential oils of *Ocimum tenuiflorum*. The major constituents of *Ocimum tenuiflorum* grown in Australia (methyl chavicol 87%), Bangladesh (eugenol 41.7% and sesquiterpene components 45.9% in green type and eugenol 77.5% in purple type), Cuba (eugenol 34.3%, β-elemene 18.0% and β-caryophyllene 23.1%), Germany (eugenol 24.2%, α-bisabolene 10.6%, β-bisabolene 15.4% and methyl chavicol 11.6%) and India (eugenol 35-53%) are distinctly different from the chemotype reported here [10-12].

Both essential oil of OT were found to exhibit antimicrobial activity and against similar microorganisms, i.e., *Staphylococcus aureus*, MRSA and *Aspergillus niger*. However, the oil extracted from the leaves collected prior to flowering was found to have more pronounced antifungal properties (MIC of 0.25 mg/ml recorded against *Aspergillus niger*) while the essential oil collected after flowering was found to have stronger antibacterial potential as depicted by their MIC values in Table **1** (0.25 mg/ml against both *Staphylococcus aureus* and MRSA). The observed activities might be due to the consequence of synergy existing between the different components of the essential oils or the annihilation of antagonism between components. The pronounce antimicrobial activity observed in essential oil of flowering season might be due to the increase in its content of both caryophyllene and δ-cadinene, which have both been reported for their antibacterial activities [10]. The higher antifungal properties of the oil derived prior to flowering was mainly due to its higher content in carvacrol and eugenol. Crude aqueous and methanolic extracts of the different parts of OT were also found to exhibit antimicrobial activity, all except aqueous extract of stem and inflorescences. The most active extract was found to be the methanolic extracts irrespective of sampling period (Table **1**). Indeed, methanolic extracts of the plant have been reported for their ability to inhibit clinical isolates of β-lactamase producing MRSA [10].

Phytochemical screening of the plant samples from either season showed that the whole plants constituted of alkaloids, flavones, saponins, terpenes, sterols, tannins and triterpenes.

However, it was observed that these secondary metabolites varied in their distribution within the plant as shown in Table **2**, which might justify the observed antimicrobial activities of OT. Anthocyanidins were detected in leaves collected prior to flowering and in inflorescence only. Flavonoids were observed in both leaves samples and also in the inflorescence. For sample collected prior to flowering, Leucoanthocyanidins were detected in leaves only, while it was observed in both leaves and stem of samples collected after flowering. Phenol was recorded in the different plant parts in the flowering season but

Table 1: Antimicrobial Activity of Essential Oils and Crude Extracts of the Different Plant Parts of *Ocimum tenuiflorum* Before and After Flowering

Samples	Plant Parts	Minimum Inhibitory Concentration (mg/ml)					
		S. aureus	MRSA	*E. coli*	*S. typhi*	*A. niger*	*C. albicans*
Essential oil before Flowering	Leaves	0.5	0.25	-	-	0.25	-
Essential oil after Flowering	Leaves & inflorescence	0.25	0.25	-	-	0.5	-
Crude aqueous extract before flowering	Leaves	8	8	-	-	-	-
	Twigs	-	-	-	-	-	-
Crude aqueous extract after Flowering	Leaves	8	-	-	-	-	-
	Twigs	-	-	-	-	-	-
	Inflorescence	-	-	-	-	-	-
Crude methanolic extract before flowering	Leaves	8	4	-	-	2	-
	Twigs	8	4	-	-	8	-
Crude methanolic extract after flowering	Leaves	8	8	8	8	-	-
	Twigs	8	8	-	-	-	-
	Inflorescence	4	4	-	-	4	-

Table 2: Phytochemical Fingerprint of the Different Plant Parts of *Ocimum tenuiflorum* Prior and After Flowering

Phytochemicals	Plant parts of *Ocimum tenuiflorum* (Prior to flowering)		Plant parts of *Ocimum tenuiflorum* (After flowering)		
	Leaves	Twigs	Leaves	Twigs	Flowers
Alkaloids	+	+	+	+	+
Anthocyanidins	+	-	-	-	+
Anthraquinones	-	-	-	-	-
Coumarins	-	-	-	-	-
Flavones	+	+	+	+	+
Flavonoids	+	-	+	-	+
Leucoanthocyanidins	+	-	+	+	-
Phenols	+	-	+	+	+
Saponins	+	+	+	+	+
Sterols/terpenes	+	+	+	+	+
Tannins	+	+	+	+	+
Triterpenes	+	+	+	+	+

was found retrained to the leaves when the plant is in the stage prior to flowering. The plant's phenology obviously had an effect on the distribution of these phytochemicals within the plant. Indeed, available reports tend to show that alkaloids (present in all the plant parts) and flavonoids are the responsible compounds for the antimicrobial activities in higher plants [5].

Based on the acute oral toxicity study, it can be concluded that a dose of 1120 mg/kg of crude extracts and 1100 mg/kg of essential oil of OT given orally was non-toxic. There was no mortality or any sign of behavioural change or toxicity observed after oral administration of both crude extracts (before and after flowering) and both essential oils (before and after flowering) at these maximum concentrations. This suggests that the LD_{50} of crude extracts and essential

oils of OT, irrespective of sampling season, were above 1100 mg/kg and considered to be safe as per OECD. The results of the current study therefore corroborate reported values for lethal doses and concur with the use of this plant in the local traditional medicine. Results from the present investigation tend to show that OT crude extracts and essential oil do not exhibit any acute toxicity. Hence decoction/infusion of the OT can be considered as generally safe and with potent antimicrobial potential.

ACKNOWLEDGEMENTS

The Univ. of Mauritius for financial support. We are also thankful to Dr. Issack, Clinical Microbiologist, Victoria Hospital, Ministry of Health & Quality of Life for providing us with clinical isolates.

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Received on 25-12-2011 Accepted on 29-02-2012 Published on 06-04-2012

DOI: http://dx.doi.org/10.6000/1927-3037.2012.01.01.06