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Compositional Analysis of Volatile Compounds of *Paspangiri* - A Mixture of Leaves of Five Rutaceae Plants Used as an Effective Remedy for Respiratory System Microbial Infections in Traditional Systems of Medicine

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Abstract Steaming with aromatic poly herbal formulations has been practiced as an effective treatment for curing or controlling infectious diseases, deterring of pest species, destroying of unwanted microbial contaminations by several cultures. Steaming of *Pasppangiri*, a mixture of leaves of five species *(Citrus aurantium* Linn, *Citrus aurantifolia* (Christm. & Panzer) Swingle, *Citrus sinensis* Linn, *Atlantia ceylanica* (Am.) Oliver, *Citrus reticulata* Blanco) has been used for an effective treatment for curing of microbial infected, respiratory system related diseases. However, information on volatile compounds, compositional analysis of *Paspangiri* is lacking or scattered. Leaves of five citrus species were collected from previously authenticated plants grown in same soil and climatic conditions. Essential oil was separated by hydro distillation method. The chemical composition of the essential oil of *Paspangiri* was analysed using gas chromatography-mass spectrometry (GC-MS). Results revealed that the number of compounds observed in each species were varied as *Citrus sinensis* (27)>*aurantium* (25) >*Citrus reticulata* (20)>*Citrus aurantifolia* (19)>*Atlantia ceylanica* (5) respectively. The major bioactive molecules identified in essential oils of Paspangiri such as linalool, limonene, caryophyllene, eugenol, z-citral, ∞- pinene, sabinene, and myrcene, either singularly or as a mixture have been exhibited antimicrobial properties. Therefore, the present study, partially validate the traditional claims of inhaling of *Paspangiri* steam for prevention or curing of infectious disease.

Keywords: *Rutaceae, citrus species, leaf essential oil, GC-MS analysis, Paspangiri, traditional medicine*

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1. Introduction

Since ancient times, fragrant materials or essential oils have been used in rituals for the curing of diseases, deterring of pest species, destroying of unwanted microbial contaminations etc. [1]. Essential oils are mixture of volatile aroma substances, produced and secreted by glandular trichrome, diffused onto the surface of plant organs, particularly flowers and leaves, to maintain their natural defense mechanism as well as to attract the beneficial organisms to ensure the survival of the plants [2,3,4,5]. Currently, essential oils and their individual constituents have become an essential part for traditional Chinese medicines (TCM), Ayurveda, aromatherapy and alternative complementary medicines (CAM) around the globe for the treatment of microbial infections and other disorders [6,7]. Many plants belonging to Rutaceae family, (Citrus family), are traditionally used for the prevention or control of infectious diseases caused by bacteria, fungus and viruses [8]. In addition, plants belonging to citrus family have been used for the treatment of many ailments in traditional systems of medicine in Sri Lanka. One of the famous and the most ancient methods is steaming of poly herbal mixture of leaves of five Rutaceae plants (*Paspangiri*) which includes leaves of *Citrus aurantium* Linn, *Citrus aurantifolia* (Christm. & Panzer) Swingle, *Citrus sinensis* Linn, *Atlantia ceylanica* (Am.) Oliver, *Citrus reticulata* Blanco and use as an effective treatment for microbial infected diseases such as common flue, hepatitis, and many other respiratory tracts related diseases (Personnel communication). Studies conducted for investigation of chemical composition of essential oils of citrus species revealed that some compounds which are present in essential oils such as citral, limonene, sabinene, and caryophyllene have proven antiviral activities [9]. However, information on compositional analysis of essential oils of above 5 species belonging to family Rutaceae is scattered. Therefore, the present study was aimed to investigate the essential oil compositions of *Paspangiri*.

2. Materials and Methods

2.1. Plant Materials

Plant materials were collected from previously authenticated plants maintained under same soil and climatic conditions. Leaves of the collected Rutaceae species were separated, washed toughly with running tap water, rinsed with distilled water to remove surface debris and air dried for two days.

Figure 1. Five Rutaceae species used for the study [A- *Citrus sinensis* L.; B- *Citrus aurantifolia* (Christm. & Panzer) Swingle; *Citrus aurantium* L.; D- *Citrus reticulata* Blanco.; and *Atlantia ceylanica* (Am.) Oliver.]

2.2. Extraction of Essential Oil

Leaves were cut into small pieces and 100 g of each plant material was mixed with 500 mL of water were subjected to hydro-distillation for 5 hours at 105°C using a Clevenger apparatus (EM 0250, Electrothermal.UK) separately. Essential oils accumulated in the apparatus were separated and dried over anhydrous sodium sulphate. Then the essential oil samples were stored in a refrigerator at + 4°C in dark until further analysis.

2.3. Gas Chromatography - Mass Spectrometry (GC-MS) analysis

The essential oils of *Paspangiri* species were analyzed by GC-MS according to method described by Ouedrhiri and co-workers [15]. GC-MS analysis were carried out using an Agilent 6890 series ultra-instrument coupled to an Agilent 5973 N series mass spectrometer equipped with an injecting split less mode and a HP-5 MS capillary column (length 30 mm, internal diameter: 0.25 mm, thickness film: 0.25 µm). The flow rate of the carrier gas (Helium) was 0.9 mL/min. A sample of 1 µL (solvent: hexane) was injected, using split less mode with an injection temperature of 250°C. The column temperature was programmed from 35°C (5 min hold) to 280°C at a rate of `5°C /min. Coupling with the Mass Spectrometer result with interface temperature 30°C. The operating conditions are as follows: Scan parameters were 15 (amu) - 550 (amu), source temperature of ionization 250°C, ion trap detector. Total run time was 54.0 min. The composition of the essential oils was reported as a relative percentage of the total peak area. The identification of the constituents was performed by comparing with the mass spectral values with the known compounds in Wiley W9N08 and NIST mass spectral search database.

2.4. Statistical Analysis

All data were analysed using descriptive statistics. Values of essential oil composition are mean value of duplicate.

3. Results and Discussions

The chemical composition of the essential oil of *Paspangiri* was analyzed using GC-MS. As demonstrated in Table 1, the highest number of compounds were identified in *C. sinensis* (Sweet orange) followed by *Citrus aurantium* (Sour orange), *C. reticulata* (Mandarin), *C. aurantifolia* (Lime) and *Atalantia ceylanica* (Ceylon atalantia) respectively. The cumulative number of compounds observed in all five species were 68 representing more than 85% of total essential oil profile. The compounds observed in each species was varied as *Citrus sinensis* (27) > *Citrus aurantium* (25) >*Citrus reticulata* (20) > *Citrus aurantifolia* (19) >*Atlantia ceylanica* (5) respectively.

The major compounds found in *Citrus reticulata* were Sabinene (25.42%), linalool (20.73%), ∞- pinene (7.37%) and β- ocimine (6.49%) while, limonene (24.58%), Z- citral (16.23%), valencene (14.36%), citronella (4.46%) and cis-geraniol (6.51%) were the major compounds found in *C. aurantifolia*. Meanwhile, *Atalantia ceylanica* exhibited the lowest number of compounds majoring, Caryophyllene (40.2%), Caryophyllene oxide (19.48%), ∞-cardinal (11.34%) and lauraldehyde (8.48%) respectively.

Among the species tested, more than 60% of oil profile of *C. sinensis* consisted of 5 major compounds, such as limonene (34.89%), linalool (10.58%), β-myrcene (9.28%), ∞-pinene (3.24%), and sabinene (2.59%), while the rest of 22 compounds only represented 40% of essential oil profile. Out of 25 compounds identified in the essential oil profile of *C. aurantium*, only 4 major compounds such as linalyl acetate (34.82%), β- linalool (24.36%), ∞-terpineol (11.05%) and nerol (6.41%) represented 75%, while rest of 21 compounds were identified as minor compounds.

Table 1. Components of the essential oils of *Paspangiri* species identified by GC-MS analysis

Compound	*Citrus reticulata Blanco*	*Citrus aurantifolia (Christm.) Swingle*	*Atalantia ceylanica (Arn.) Oliv.*	*Citrus sinensis* (L.) *Osbeck*	*Citrus aurantium* L.
∞-Thujin	0.51				
∞- Pinene	7.37			3.24	
Sabinene	25.42			2.59	0.27
β-pinene					0.49
β-myrcene	3.49	1.12		9.28	1.31
Octanol				0.96	
Limonene	3.34	24.58		34.89	
DL limonene					0.49
Limonene oxide				0.75	
∞-terpinolene	1.07			1.21	
P cymene	3.66				
β- terpineol	0.27				
β- ocimine	6.49	2.08			1.95
Cis ocimine		0.63			0.84
Capra					
Decanal			6.43		
γ- terpinene	2.17				
Trans sabinene hydrate	2.34				
Linalool oxide					0.73
β- linalool		1.93			24.36
Linalool	20.73			10.58	
Linalyl acetate					34.82
Lauraldehyde			8.48		
Camphene					0.22
Citronella		4.46			
Citronella				1.26	
Nerol				0.39	6.41
∞- terpineol				2.13	11.05
4-terpinol					0.20
Benzoic acid	1.03				
∞- terpinolene	5.45				0.65
β- fenchyl alcohol				2.46	
Carveol				1.31	
Elemol		3.76			
Neryl acetate					3.94
Valencene		14.36			
Z-citral		16.23		1.63	
E- citral				1.91	
∞- citral	0.7				
Citranolic acid		0.76			
β- gurjune		4.26			
Cis geraniol		6.51		1.59	2.61
Caryophyllene oxide	2.1	5.28	19.48		
Merhyl benzoate				0.98	
Caryophyllene			40.2		
Caryophyllene oxide				1.83	3.46
β- farnesene					1.08
β-4- dimethyl		1.02		0.41	
∞- humulene		0.77		0.43	0.53
Germacrene D		2.02		0.28	
Spathulenol					0.24
Nepthalene					0.24
Humulene oxide		0.54			
β- bisabolene	0.46				
δ- cardenene				0.37	0.21
∞- eudesmol	0.29				
Nerolidol					0.47
∞- bisabolol		0.8			
γ- eudesmol				0.26	
γ- muurolene				0.35	
∞- cardinol			11.34		0.2
Eucarvone	0.32	0.61			
Phytol					1.24
1-naphthalenamine	0.31				
γ- muurolene	3.23				
∞- sinensal				0.15	
β- sinensal				0.28	
Hexadecanoid acid				0.77	
Total	90.69	91.72	85.93	82.29	80.29

According to the WHO, traditional medicine has been identified as the use of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, used for prevention, diagnosis, improvement or treatment of physical and mental illness. Similarly use of poly herbal mixtures for controlling, curing or prevention of infectious diseases is a common practice in traditional systems of medicine in Sri Lanka. Steam inhalation of *Paspangiri;* a collection of leaves of five Rutaceous species is one of the such poly herbal treatments, widely used in respiratory system related microbial infections in Sri Lankan traditional systems medicine. However, information on composition of steam of *Paspangiri* yet to be verified. Therefore, in the present study, essential oil composition of leaves of five citrus species, which were used as a poly herbal preparation (*Paspangir*i) for the control or prevention of viral infections in traditional systems of medicines in Sri Lanka were analyzed. Since all samples were obtained from the previously authenticated, plants which were grown under same soil and climatic conditions, the obtained results depict the reliable chemical profiles. Even though all tested plants were belonging to Rutaceae family, their chemical profiles exhibited a heterogeneous group of compounds. Leaf essential oil of all tested materials of *Paspangiri* possessed approximately 68 bioactive molecules and the major compounds identified were limonene, linalool, linalyl acetate, β-myrcene, sabinene, Z- citral, *caryophyllene, caryophyllene oxide,* ∞- cardinol and valencene. Previous studies reported that the major compounds present in essential oil of *C. sinensis* were limonene, followed by sabinene, linalool, myrcene, and ∞- pinene respectively [6,10,11]. Moreover, presence of β- linalool, linalyl acetate, ∞- terpineol, Caryophyllene and neryl acetate as major compounds of *Citrus aurantium* are in agreement with Tao et al., [12]. Further, the results of the present study, on composition of leaf essential oil of *C. aurantifolia* are also in agreement with previous studies which reported limonene, geraniol, and citral as major compounds available in *C. aurantifolia* leaf essential oil [13,14] However, our results of caryophyllene, cardinol, lauraldehyde and caryophyllene oxide as major compounds identified in leaf essential oils of *A. ceylanica*, has not been reported previously.

The promising antimicrobial bioactive compounds found in *Paspangiri* steam were linalool, limonene, caryophyllene, eugenol, z-citral, ∞- pinene, sabinene, and myrcene. The most of these compounds either singularly or as a mixture have been exhibited strong anti-bacterial, anti-fungal and also antiviral activities [6,13,14,15]. Further, inhalation of hot air steam with antimicrobial bioactive molecules present in essential oils of *Paspangiri* may help in one hand to loosening the mucus in the nasal passages, throat, and lungs and on the other hand, to slow down or inactivate the microbial action on mucus membrane of respiratory system. Therefore, the findings of present study, partially validate the traditional claims of steaming of *Paspangiri* for infectious disease.

4. Conclusion

Five ingredients used for *Paspangiri* are *C. sinensis* (sweet orange), *Citrus aurantium* (sour orange), *C. reticulata* (mandarin), *C. aurantifolia* (lime) and *Atalantia ceylanica* (Ceylon atalantia). The number of major chemical compounds was 68 representing *Citrus sinensis* (27), *aurantium* (25), *Citrus reticulata* (20), Citrus *aurantifolia* (19), Atlantia *ceylanica* (5) respectively. The major bioactive anti-microbial molecules present in essential oils of *Paspangiri* were linalool, limonene, caryophyllene, eugenol, z-citral, ∞- pinene, sabinene, and myrcene. Based on the results, present study, partially validate the traditional claims of steaming of *Paspangiri* for infectious disease. However, for the confirmation of these results, it is essential to conduct clinical trials and confirm the amount should be taken, the level of humidity, temperature, duration of steaming, frequency of steaming and also to observe for any allergy interactions.

Conflicts of Interests

Authors declare that there are no conflicts of interests.

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