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Characterization of essential oils from four different indigenous citrus varieties of Northeast India and their antioxidant activities

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Abstract

The characterization of the essential oils of four different varieties of citrus species were analysed by GCMS. The results showed that the monoterpene hydrocarbons were most abundant chemical compounds with 76.66%, 70.99%, 78.47% and 46.66% in essential oils of *Citrus jambhiri* , *Citrus medica*, *Citrus limon* (elongated) and *Citrus aurantifolia* (rough lemon) respectively. The limonene was the major compound identified in all the tested oils representing 72.901% in *Citrus jambhiri*, 67.861% in *Citrus medica*, 70.109% in *Citrus limon* (elongated) and 36.386% in *Citrus aurantifolia* (rough lemon). From DPPH assay, all the oils showed significant DPPH scavenging capacity at different percentages of oils and antioxidant activities increased with increased in percentages of oils.

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Introduction

The north east India is rich in diverse varieties of Citrus in the form wild and semi wild distributed throughout the region. The diverse varieties of citrus species grow due to edaphic, climatic and physiological conditions of the region. The citrus varieties are known in the region due their dietary, nutritional, good source of citric acid and phenolic compounds (T.K. Hazarika, 2012). Citrus varieties are belongs to Rutaceae family having approximately 140 genera and 1300 species that mainly grow in tropical and subtropical region of the world. The large amount of wastes is produced every year from citrus processing industries and fresh consumption. The peels of citrus species are rich in essential oils so the extraction of essential oils and utilize them in various applications is the ways to reduce peel wastes. The essential oils of citrus are have diverse chemical components and activities like antimicrobial, anticancer and insecticidal etc. The essential oils have considerable antioxidant activities and can be used in food and pharmaceuticals industries as natural antioxidant (Xiaocai Lin *et al.*, 2021). The essential oils have various classes of terpenoids and phenolic compounds. Recent times various studies on essential oils are being carried out due their antimicrobial and antiviral properties (A.K Pandey *et al.*, 2017). Plant derived essential oils may be used as food preservatives and are called as GRAS (Generally Recognised as safe) due their inhibitory activities against foodborne pathogens. Citrus essential oils are mainly located in peels and their extraction is considered sustainable, because peels are wastes in juice industries. Due to inhibitory activities of citrus essential oils against microbes are used in industries like food, beverages, cosmetics and medicines (O Koul *et al.*, 2008). The main components of essential oils are low molecular weight compounds like terpenoids and aromatic phenols. The other components that may present are fatty acids, derivative of sulphur and oxides (K Winska *et al.*, 2019; SK Yang *et al.*, 2021). The drug resistance, toxicity and environmental hazards the use chemical antimicrobials are restricted and the ability of essential oils to manage drug resistant pathogens are useful for preventing spread of several diseases (MK Swamy *et al.*, 2016). The antimicrobial activities of essential oils depend on chemical compositions. The essential oils mainly effects cellular membrane thereby creates pores. This leads to disturbance in the metabolic activities like energy production and membrane transport but their effects on gram positive and gram negative are different due to varied chemical compositions of their plasma membrane (A.K Pandey *et al.*, 2017 ; ML Lota *et al.*, 2002). Hence this present study mainly focuses on the diverse chemical composition of essential oils of four different varieties of citrus species of north east India and their antioxidant activities to explore them as natural antioxidant in different industries like food, cosmetics and pharmaceutical etc.

Material and methods

Collection of four citrus varieties of north east India

The four different varieties of citrus varieties of north east India namely *Citrus jambhiri* (Sample code PL1), *Citrus medica* (Sample code PL2), *Citrus limon* (elongated, Sample code PL3) and *Citrus aurantifolia* (rough lemon Sample code PL4) were collected from local market Guwahati, Assam.

Extraction of essential oils

Extraction of essential oils was done by slight modification of method described elsewhere (A.A Mohamed *et al.*, 2013). Briefly, fresh lemon fruit peels were divided into small pieces, weighted, and hydrodistilled for 4 hours in a Clevenger-type equipment to extract the EOs. 500 ml of water and 100 g of sample were added to a flask with a circular bottom before being put into a Clevenger-style device. After 4 hours of boiling, the sample was collected in a centrifuge tube.

Characterization of EOs by GCMS analysis

The chemical composition of the EOs were analyzed by injection 1 µl of EOs in GC-MS system (Perkin Elme, USA, Model: Clarus 680 GC & Clarus 600C MS comprising a liquid auto-sampler) equipped with Elite-5MS capillary column. The interpretation of the peaks in GC chromatogram was done by library search of the mass spectrum of the corresponding

peaks using database software of NIST-2014. The name, molecular weight and empirical formula were determined by comparing the mass spectrum of the unknown components with the known components of NIST library.

Antioxidant activities of the EOs

The antioxidant activities of the EOs were evaluated with some modification of the method described elsewhere (RPP Fernandes *et al.*, 2016). Briefly 10 µl of EOs of different concentrations were added to 190 µl of 60 µM DPPH solutions and mixed thoroughly. And then kept the mixed content in dark for 30 mins at room temperature. The different concentrations of ascorbic acid (0.25 to 1.0 mg/ml) were used as positive control while the reaction mixture with methanol in place of EOs was used as negative control. Then absorbance was taken at 517 nm and DPPH radical scavenging activity was calculated by formula:

$$\% \text{ DPPH scavenging activity} = (O.D_{control} - O.D_{test}) / O.D_{control} X_{100}$$

Result and discussion

GCMS analysis of the oils of citrus varieties of north east India Total 60 compounds were identified in the essential oils of four different varieties of the citrus species. According to table 1 monoterpene hydrocarbons are major parts in the essential oils PL1, PL2, PL3 and PL4 having 76.66%, 70.99%, 78.47% and 46.66% respectively. These findings are accordance with the previous finding of (ML Lota *et al.*, 2002) in which they showed high level of monoterpenes.

Table 1. The chemical compositions and their abundance of EOs of PL1, PL2, PL3 and PL4 respectively.

Compound name	RT	LP1	LP2	LP3	LP4
Monotarpene hydrocarbon			Area %		
Alpha-Pinene	12.220	3.222	0.494	0.570	2.527
Limonene	13.516	72.901	67.861	70.109	36.386
Gamma-Terpinene	12.310				6.249
Fenchol	14.906				0.173
Cis-Verbenol	15.441	0.136			
2-Carene	19.218	0.323			1.001
Limonene oxide	15.171	0.081			
Beta-Myrcene	12.120		2.631		
Alpha-Phellandrene	11.115				0.326
Beta-Pinene	12.120			2.747	
Trans-Verbenol	15.466			3.029	
Carveol	17.197			2.012	
		76.663	70.986	78.467	46.662
Hemiterpene					
Prenol	8.158				0.014
					0.014
Diterpenes					
Neophytadiene	18.427	0.313		0.384	
Trans-Geranylgeraniol	15.536		0.285		
		0.313	0.285	0.384	
Triterpenoid					
Squalene	26.366			0.074	
				0.074	
Sesquiterpenes					
Alpha-Farnesene	21.179		1.08		2.365
Caryophyllene	21.139	3.372	0.831	2.197	

Beta-Bisabolene	22.544	2.502	1.855		
Longifolene	22.579			2.809	2.594
Cis-Alpha-Bergamotene	21.199			1.083	
Alpha-Bulnesene	21.179				1.16
Humulene	23.059			0.077	0.135
Alpha-Bisabolol	25.675		0.377	0.391	0.333
Gamma-Himachalene	24.825				0.234
Globulol	24.565	0.056			
Caparratriene	25.510	0.098			
Nerolidol	23.349		0.039		
Caryophyllene oxide	24.170			0.021	
Phytol	31.623			0.014	
		6.028	4.182	6.592	6.821
Saturated hydrocarbons					
Cyclododecanol	16.377	1.768			
		1.768			
Unsaturated hydrocarbons					
Cyclopentadiene	12.255				8.55
4-Undecene, 6-Methyl	15.611	0.136			
2,4,6-Octatriene, 2,6-Dimethyl	14.816				
3,7,11-Trimethyl-3-hydroxy-6,10-Dodecadien-1-yl acetate	23.319	0.028			
Cyclohexene,1-Methyl-4-(1-Methylethylidene)	14.206				1.449
		0.164			9.999
Oxygenated Monotarpene					
Linalool	14.316	0.432			
Citronellal	15.301	0.707	7.267	3.029	0.129
Citronellol	16.837	1.665	8.038	2.395	2.844
Geraniol	17.357	1.186			1.404
Geranic acid	9.739				0.242
Alpha-Terpineol	16.522				4.577
Terpinen-4-ol	16.092	0.069			3.286
		4.059	15.305	5.424	12.482
Aldehydes					
Nonanal	14.381	0.475	0.408	0.616	
Citral	17.762	2.367		2.62	2.157
Decanal	16.402		1.665	1.448	
Undecanal	18.447		0.28		
Hexadecanal	24.215		0.094		
2-Octenal	9.684	0.034			
Isoneral	15.811	0.177			
3,6-Octadienal, 3,7-Dimethyl	15.101	0.086			
		3.139	2.447	4.684	2.157
Esters					
Geranyl Palmitoleate	19.373	0.22			
Lavandulyl propionate	19.763	0.316		0.302	
Citronellyl oleate	19.233		1.06		
Tricosenyl formate	27.511		0.127		

Z-10-Methyl-11-Tetradecen-1-ol propionate	27.471	0.087			
		0.623	1.187	0.302	00
Organic acid and Phenol					
Methoxyolivetol	25.645	0.686			
3,4-Dimethylbenzyl alcohol	13.796				7.688
Oleic acid	19.488		0.009		
4-Chloro-2-Methoxybenzyl alcohol	26.411		0.025		
		0.686	0.034		7.688

RT: Retention time.

The other components identified in the essential oils are oxygenated monoterpene, sesquiterpenes, diterpene, Triterpenoid, hemiterpene, aldehydes, saturated hydrocarbons, unsaturated hydrocarbons, esters and organic acid and phenol etc. For essential oil PL1, total of 27 compounds were identified by GCMS analysis. The main chemical compositions of the oil are limonene (72.901%), alpha- pinene (3.222%), caryophyllene (3.372%), Beta-Bisabolene (2.502%), Cyclododecanol (1.768%), Citronellol (1.665%), and Geraniol (1.186%). The abundance of the other components is less than 1%.

The total of 19 compounds was identified in the essential oil PL2. The major compounds are limonene (67.861%), Beta-Myrcene (2.631%), Alpha-Fernesene (1.08%), Beta-Bisabolene (1.855%), Citronellal (7.267%), Citronellol (8.038%), Decanal (1.665%) and Citronellyl oleate (1.06%). The other compounds identified have abundance less than 1%. By GCMS analysis total 20 compounds were identified in the oil PL3. The major abundant chemical compounds are limonene (70.109%), Beta-Pinene (2.747%), Trans-Verbenol (3.029%), Carveol (2.012%), Caryophyllene (2.197%), Longifolene (2.809%), Cis-Alpha-Bergamotene (1.083%), Citronellal (3.029%), Citronellol (2.395%), Citral (2.62%) and Decanal (1.448%). The chemical compounds identified were less in abundance. Similarly 23 compounds were identified in oil PL4. The major abundant compounds are limonene (36.386%), Alpha-Pinene (2.527%), Gamma-Terpinene (6.249%), 2-Carene (1.001%), Alpha-Farnesene (2.365%), Longifolene (2.594%), Alpha-Bulnesene (1.16%), Cyclopentadiene (8.55%), Cyclohexene,1-Methyl-4-(1-Methylethylidene (1.449%), Citronellol (2.844%),Geraniol (1.404%), Alpha-Terpineol (4.577%),Terpnen-4-ol (3.286%), Citral (2.157%), 3,4-Dimethylbenzyl alcohol (7.688%).and the other chemical compounds identified were less than 1%. Our findings proved that essential oils composed of many diverse compounds which determine the various biological activities of the oils. Hence for improvement of the biological activities, the characterization of essential oils of citrus species is necessary.

Antioxidant activities by DPPH assay

All tested essential oils of four different varieties of citrus species of north east India assayed for DPPH scavenging capacities and reported increase in antioxidant activities. The antioxidant activities of the essential oils of the four different varieties of citrus species at 1% (v/v) found to be 44.70 ±.44% for PL1, 44.38± 0.07% for PL2 and 52.95± 0.15% for PL3 and 42.75± 0.34% for PL4. The antioxidant activities for the oils (PL1, PL2, PL3 and PL4) at 2% (v/v) found to be 48.71±1.0%, 67.06±0.01%, 58.55±0.67% and 49.02±.15 % respectively. Similarly at 4 %(v/v) antioxidant activities of the oils were 72.98±0.87%, 71.81±0.10%, 65.86±0.11% and 70.32±0.07%.

The antioxidant activities of all the oils were also assayed at 8 %(v/v) and percentage of DPPH scavenging capacity found to be 73.81±0.06% (for PL1), 74.92±0.02% (for PL2), and 74.18±0.04% (for PL3) and 74.88±0.07% (for PL4). Similarly the antioxidant activities of the oils at 16 %(v/v) found to be 76.09±0.03% (for PL1), 77.54±0.05% (for PL2), 77.76±0.07% (for PL3) and 76.20±0.03% (for PL4). All the oils have DPPH scavenging capacities ranging from 44% to 77% which is in accordance with the finding of (Wei *et al.*, 2007).

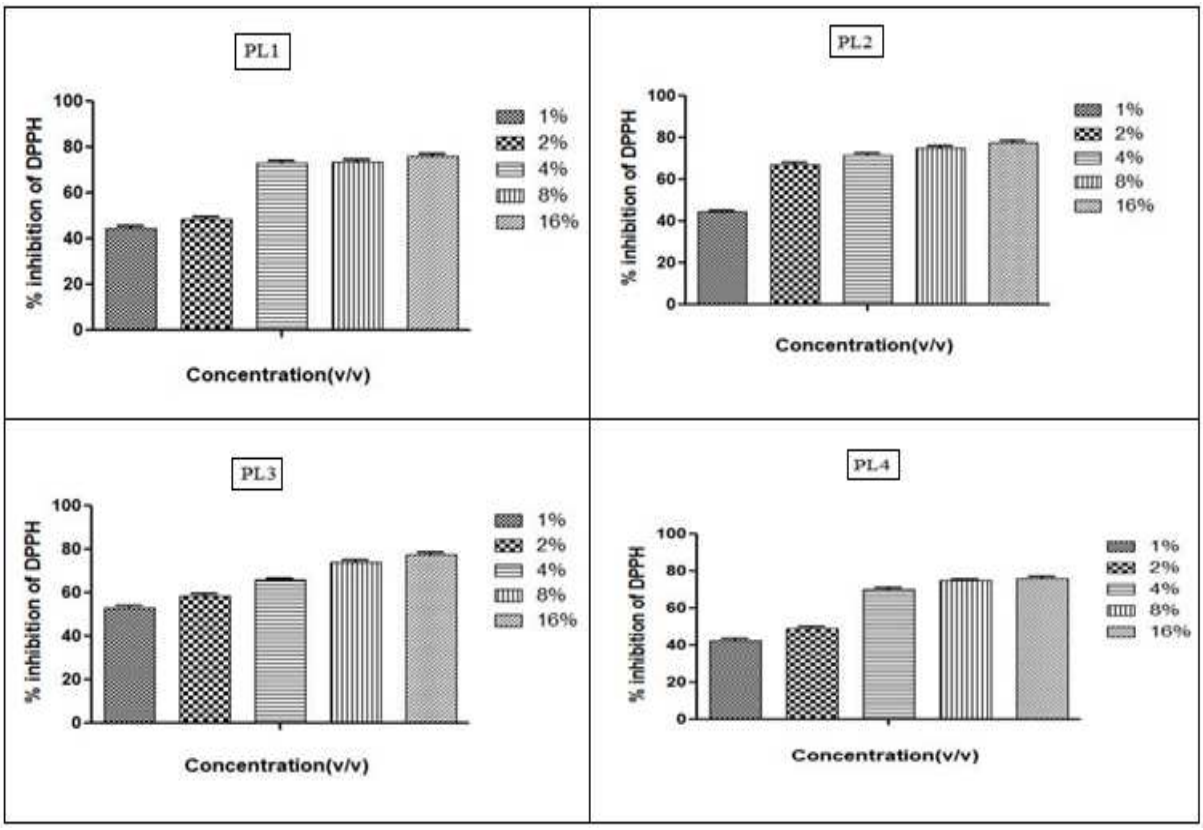

Fig. 1. Antioxidant activities of the EOs of four citrus varieties of north east India.

The good antioxidant activities of the essential oils may be related with the higher abundance of the monoterpene hydrocarbons mainly the limonene which is abundant in all the analysed essential oils of four different varieties of citrus of north east India and reported to have good antioxidant activity. In conclusion, our findings provide a confirmation that oils of citrus varieties can be used as natural antioxidant in different industries like food, cosmetics and pharmaceuticals etc.

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