Comparative studies of essential oil of fruit peels of four citrus species (family: Rutaceae) in Nigeria

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Comparative studies of essential oil of fruit peels of four *citrus* species (family: Rutaceae) in Nigeria

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Abstract
Comparative GCMS analysis was carried out on the essential oils of the fruit peels of four *Citrus* species: *Citrus sinensis* (Linn) Osbeck (Sweet orange), *Citrus Limon* (Linn) Osbeck (Lemon), *Citrus paradise*. Mactad (Grape fruit), and *Citrus Aurantifolia* (Christin) Swingle (Lime). *Citrus Limon* had 28 compounds identified, *Citrus sinensis* and *Citrus paradise* had 26 compounds each and *Citrus aurantifolia* had 21 compounds. The compounds were classified into monoterpenes, alcohols, aldehydes, ketones, esters, sesquiterpenes, oxides and other compounds. Monoterpenes were the predominant constituents in all the species studied and accounting for 72.83%, 68.75%, 57.19% and 49.35% for *C. paradise*, *C. sinensis*, *C. aurantifolia* and *C.* Limon respectively. Alcohols constituted 22.28% (*C. aurantifolia*), 18.57% (*C. sinensis*), 18.23% (*C.* Limon) and 12.23% (*C. paradise*). Aldehydes were 7.53% (*C.* Limon), 5.83% (*C. sinensis*), 5.62% (*C. paradise*) and 4.45% (*C. aurantifolia*). Sesquiterpenes gave 11.62% (*C.* Limon), 4.81 (*C. paradise*), 2.80 (*C. aurantifolia*) and 1.34% (*C. sinensis*). Oxides had 2.75% (*C. aurantifolia*), 2.50% (*C. paradide*), 1.38% (*C.* Limon) and 0.38% (*C. sinensis*). Ketones had 3.78% (*C.* Limon), 2.61 (*C.* aurantifolia), 2.21% (*C. sinensis*) and 0.00% (*C. paradise*). Esters had 3.97% (*C.* Limon), 0.91% (*C. aurantifolia*), 0.74% (*C. sinensis*) and 0.00% (*C. paradise*). Other components had 6.76% (*C. Limon*), 4.13% (*C. aurantifolia*), 1.35% (*C. paradise*) and 1.20% (*C. sinensis*). The major compounds found in all the *Citrus* species were: Limonene, alpha terpineol, alpha pinene, beta-pinene, beta-linalool, lemonol, alpha and beta citral. These various bioactive constituents idenfied in *Citrus* species have a wide spectrum of applications in the pharmaceutical industry and as flavoring agents in the manufacture of perfumes and cosmetics and as flavoring agents in the manufacture of perfumes and cosmetics.

Keywords: *citrus* species, chemical components, essential oil, Nigeria

Introduction

Citrus is a genus of flowering trees and shrubs in the family Rutaceae. Plants in the genus produce *Citrus* fruits, including important crops such as oranges, lemons, grapefruits, and limes. The most recent research indicates an origin in the Himalayas. Previous research indicated an origin in Southeast Asia, bordered by Northeast India, Burma and the Yunnan province of China and it is in this region that some commercial species such as oranges, mandarins, and lemons originated. *Citrus* fruit has been cultivated in an ever-widening area since ancient times. The economic importance differs among species and compositional studies of the main species are more frequent (Young, 1986) [1]. Because of their high economic importance, numerous studies have investigated the chemical composition of the peel, leaf, and flower essential oils of different *Citrus* species. It is worth noting that there is a great variation in the chemical composition of *Citrus* oils due to differences in origin, genetic background, season, climate, age, ripening stage, method of extraction (Noura, 2003) [2]. *Citrus* waste is a valuable source of *d*-Limonene, flavonoids, carotenoids, dietary fibers, soluble sugars, cellulose, hemicellulose, pectin, polyphenols, ascorbic acid, methane and essential oils (EOs). Interestingly, the essential oil (EO) is the most vital by-product of *citrus* processing. *Citrus* EOs are broadly used as natural food additives in several food and beverage products because they have been classified and generally recognized as safe (GRAS). Furthermore, *Citrus* EOs are used as natural preservatives due to their broad spectrum of biological activities including antimicrobial and antioxidant effects. The presence of terpenes, flavonoids, carotenes, and coumarins is thought to be responsible for the strong anti-oxidative and antimicrobial activities. Due to their pleasant refreshing smell and rich aroma, *Citrus* EOs are also used in air-fresheners, household cleaning products, perfumes, cosmetics, and medicines (Wikipedia). There are several applications of citrus essential oils. EOs are used for flavouring in food and are accepted by the Food and Drug Administrations (FDA) as additives in certain type of foods. Citrus oils are used for food and beverages and they are generally recognized as safe (GRAS), being a good starting point for the use of EOs as antimicrobials within the food industry. Recently, a study was carried out to evaluate the inhibitory potential of EOs extracted from the fruit peel of several Citrus varieties cultivated in Sicily against the most common food borne pathogen bacteria (Settanni, *et al*., 2012) [3]. The antimicrobial properties, due to the phenolic compounds recognized as bioactive components with antimicrobial activity (Velázquez, *et al*., 2013) [4], combined with the aromas and flavours of EOs, led to research into their uses as potential food preservatives and the ability of citrus oils to delay spoilage and add organoleptic qualities in food products may be interesting from a commercial point of view (Bajpai, *et al*., 2012) [5]. However, because of the interactions (synergisms and antagonism) between EOs and food ingredients or additives, studies aimed to maximize the antibacterial activity of EOs and to minimize the concentrations required to achieve a particular antibacterial effect are needed. In addition, the stability of EOs during food processing should also be investigated. Dias *et al*. (2013) [8] also used EOs for developing a low density polyethylene flavouring films imbued with and/or lemon aroma. They showed that the films had a lower elongation due to the incorporation of active agents, combined with a reduction of tensile strength over time. In addition, the combined use of EOs and aroma did not affect the water vapour permeability value, but regarding colour, flavouring films had a more yellow colour and were opaque. However, application of EOs in food packaging industry of biscuits was greatly appreciated by consumers (Burt, 2004) [6]. Current chemical, medical and pharmacological literature suggests that EOs can be successfully used in many aspect of health care. Several studies indicated that EOs have antitumor effects and that monoterpenes possess a chemo preventive action against rodent mammary, skin, liver, lung and for stomach cancers (Crowell, 1999) [9]. Particularly, citrus EOs seems to contain different terpenes with antitumor effects. Additionally, among substances found in nature, terpenes constitute the main chemical class compound with antiulcer genic activity (Lewis and Hanson, 1991) [10]. Tests carried out on animals have proven the effectiveness of Limonene against some types of cancer including gastric, mammary, pulmonary adenoma and liver. Limonene has also been shown to be effective in relieving gastro oesophageal reflux disorder and occasional heartburn but the action mechanism has not been elucidated (Wilkins, 2002) [12]. For instance, a study by Lim, 1863 determined the cytotoxic effect of C. aurantifolia EOs peels against colorectal cancer, cell line, suggesting some potential antitumor effect treatments of colorectal cancer and other cancers (Odeh and Abdulkadir, 2012) [13]. The EOs extracted from Citrus lemon (lemon essential oil) was found to induce various behavioural responses in both humans and animals. In humans it was found to have antidepressant effects and, in addition, able to improve creativity, mood and perceived health (Knasko, 1992) [14]. In an ecological context, monoterpenes and sesquiterpene play an important role in the relations between organisms, for example, as attractants of pollinators or deterrents of herbivores. On a large-scale, monoterpenes emissions from vegetation in nature can have ecosystem-wide influences. Chizzola (2013) [15]. Monoterpenes are often partially responsible for the essence or odor of plants and are the major odoriferous compounds of many flowers and fruits. Seigler (1998) [16]. Sesquiterpenoids, and specifically sesquiterpene lactones from Asteraceae, may play a highly significant role in human health, both as part of a balanced diet and as pharmaceutical agents, due to their potential for the treatment of cardiovascular disease and cancer. Studies of folk medicines implicate sesquiterpene lactones as the active ingredient in many treatments for other ailments such as diarrhea, burns, influenza, and neurodegradation. In addition to the anti-inflammatory response, sesquiterpene lactones have been found to sensitize tumour cells to conventional drug treatments (Martin *et al*., 2013) [17]. Esters are used in the manufacturing of soap, the hydrolysis of fats or oils (triglyceride ester) in the presence of a strong alkali, such as (NaOH) or (KOH) to produce glycerol and (sodium or potassium salt of fatty acid) is known as saponification process which is the main reaction in the manufacture of soap. By the repetition of the condensation process, a very long molecule, which is called polyester is formed, since Dacron is an inert polymer, so, it is used to substitute the spoiled arteries and in the manufacture of artificial heart valves. Esters are used in manufacturing of textiles because the esterification of terephthalic acid with ethylene glycol produces polyester which is used in Dacron fibres industry (Martin *et al*., 2013) [17]. Organic esters are used in the manufacture of many drugs such as Aspirin and Marookh oil, the most common and simplest one is aspirin, oil of wintergreen (Marookh oil) is used as a local oil absorbed by the skin to decrease the pains of rheumatism, The acid which is used in the manufacture of these two drugs is salicylic acid, its molecule contains both the carboxylic and hydroxyl groups, It reacts as an acid or as an alcohol (Heba, 2018) [18]. Aldehydes and ketones find application in different sectors such as pharmaceutical, food, fragrance, cosmetics because of their chemical properties. Formaldehyde is found in the gaseous form. However, formaldehyde with 40% solution in water forms formalin. Formalin helps in the preservation of biological specimens. Formaldehyde reacting with phenol forms a compound Bakelite. Bakelite finds its application in plastics, coatings, and adhesives. The compound- formaldehyde is essential during many industrial processes such as embalming, glue preparation, tanning and manufacture of polymeric products. It acts as germicide, insecticide, and fungicides. Formaldehyde helps in the testing of drugs. It is also used in photography. Production of acetic acid and pyridine derivatives is possible from the compound “acetaldehyde.” Benzaldehyde (aldehyde) is an essential component for the production of perfumes, cosmetic products, and dyes. It is added to incorporate almond flavour into various food products. It also acts as a bee repellant. Ketone behaves as an excellent solvent for certain types of plastics and synthetic fibres. Acetone act as a paint thinner and a nail paint remover. It also is used for medicinal purposes such as chemical peeling procedure as well as acne treatments. Butanone, also known as methyl ethyl ketone, is one of the common solvents. It is used in textile production, varnishes production, paint remover production, paraffin wax production, plastic production, etc. Another important ketone is cyclohexanone which is an important component in nylon production. Aldehydes and ketones impart some very characteristics fragrance in compounds. For instance, ketones help in the formation a compound “acetophenone” which is responsible for fragrances such as cherry, jasmine, honeysuckle, almond, strawberry, etc. However, aldehydes are a well-known source of perfumes and fragrances in comparison to ketones. Aldehydes are an essential component in particular sugars. Moreover, certain popular flavours of baking such as cinnamon, vanilla during baking are because of aldehydes. Aldehydes also play an important part in caramelization of sugars. During the process of caramelization, amino acids convert the aldehyde group present in sugar into unsaturated aldehyde thereby helping in the formation of caramel and the characteristic brown colour of caramel. Aldehydes are also present in herbs such as coriander and give it the characteristic smell of coriander. Mixtures of volatiles and EOs isolated from plants represent the 'essence' or odoriferous constituents of the plants. They have been used since early days because of pharmaceutical properties and also as flavouring agents in the manufacture of perfumes and cosmetics. Maruzzella *et al*., 1960 [19], extensively studied the potential of EOs as antimicrobial agents in perfumes and cosmetics or against wood pathogens. The aim of this study is to compare the essential oil extracted from the fruit peels of four Citrus species, (Citrus sinensis, Citrus Limon, Citrus paradise and Citrus aurantifolia) using GC-MS analysis.

Materials and Method

Sampling, sample preparation and extraction

Fruits of the four species studied were bought from Gowon barracks on the 3rd February 2019 separately and peeled. The fruits were identified at NIPRD Herbarium. Extraction of the oil was by hydro distillation of the peels using a Clevenger apparatus.

GCMS analysis of the essential oils

The essential oils was analyzed by GC-MS using Shimadzu QP-2010 GC with QP-2010 Mass Selective Detector [MSD, operated in the EI mode (electron energy=70 eV), scan range of 45-700 amu, and scan rate of 3.99 scans/sec], and Shimadzu GCMS solution data system. The Gas chromatography column was Optima-5 ms fused silica capillary with 5% phenyl-methyl polysiloxane stationary phase, with length of 30 m, internal diameter of 0.25 mm and film thickness of 0.25 µm. The carrier gas was helium with flow rate of 1.61 mL/min. The program used for Gas chromatography oven temperature was 60- 160°C at a rate of 15°C/min, then held at 160°C for 2 min, followed by 160-280°C at a rate of 10°C/min, then again held at 280°C for 2 min. The injection port temperature was 250°C while detector temperature was 280°C. Diluted sample (1/100 in hexane, v/v) of 1.0 µL was injected using auto sampler and in the split mode with ratio of 20:80. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST 11). The percentages of each component are reported as raw percentages based on the total ion current without standardization (Okhale *et al*., 2018) [20].

Results

The chromatograms of the GCMS analysis of the four species studied are in Figures 1-4 while the various compounds analyzed are in Table 1. *C. Limon* has the highest number of compounds (28), *C. paradise* and *C. sinensis* had 26 compounds each and *C. aurantifolia* has 21 compounds. The major components of the Nigerian *Citrus* species are in Table 2.

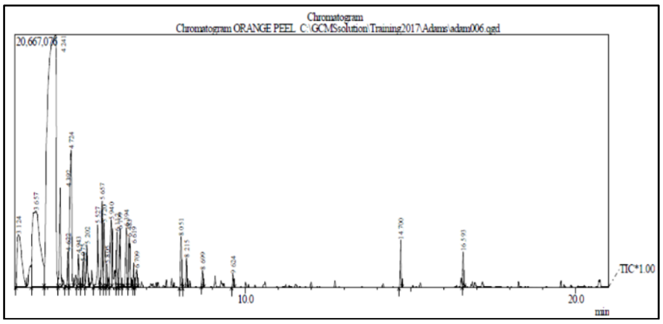

Fig 1: Chromatogram of essential oil from *Citrus sinensis* fruit peel

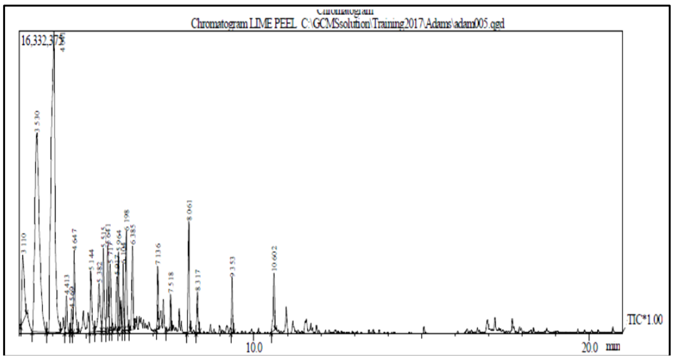

Fig 2: Chromatogram of essential oil from *Citrus aurantifolia fruit* peel

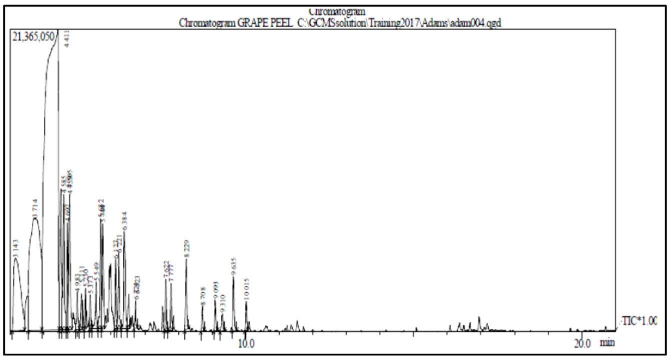

Fig 3: Chromatogram of essential oil from *Citrus paradise* fruit peel

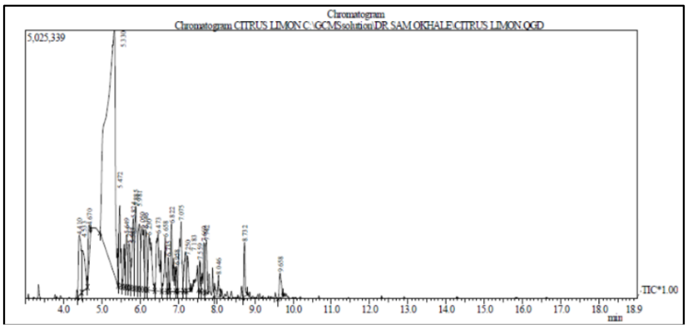

Fig 4: Chromatogram of essential oil from *Citrus Limon* fruit peel

Table 1: Chemical composition of *citrus* species fruit peels from Nigeria

Names of compounds	Retention Time	% Composition			
		C. aurantifolia	*C. paradise*	*C. sinensis*	*C. Limon*
Monoterpenes					
alpha.-Pinene	3.110	4.32	6.65	5.53	3.51
beta.-Pinene	3.530	23.65	15.81	13.56	-
Limonene	4.041	29.22	50.37	48.68	42.21
2-Carene	4.622	-	-	0.80	-
3-Carene	4.670	-	-	-	1.34
cis-Ocimene, 8-oxo-	6.523	-	0.66	-	-
Bicycle[3,1,1]hept-ene,26,6-tri	-	-	-	-	2.29
Sub total		57.19	72.83	68.57	49.35
Alcohols					
n-Octanol	4.392	-	-	2.92	-
Nonyl	5.373	-	0.42	-	1.40
Caprylic	4.505	-	3.14	-	-
beta.-Linalool	4.647	3.41	-	6.38	2.77
Linalool	4.759	-	2.74	-	-
trans-p-Mentha-2,8-dienol	4.983	-	0.61	0.73	-
(-)-4-Terpineol	5.515	3.04	0.82	-	-
4-Carvomenthenol	5.527	-	-	1.67	2.82
alpha.-Terpineol	5.641	2.79	1.83	2.11	3.62
Cis-beta- Terpineol	5.583	-	-	-	0.99
(-)-Myrtenol	5.717	2.77	-	-	-
cis-Carveol	5.917	1.86	-	2.40	-
cis-Geraniol	5.964	2.41	-	1.37	-
Lemonol	6.198	3.68	-	-	-
trans-Geraniol	6.221	-	1.32	-	3.20
cis-p-Mentha-2,8-dien-1-ol	5.111	-	0.52	-	-
L-pinocarveol	5.144	2.59	-		-
Ledol	6.658				1.73
p-Mentha-1(7),8(10)-dien-9-ol	6.619	-	-	0.43	-
p-Mentha-1,8-dien-7-ol	6.709	-	-	0.56	-
(4-Isopropenyl-1-cyclohexen-1-yl)methanol	6.723		0.36	-	-
Thunbergol	7.559	-	-	-	0.74
Lanceol, cis	8.046	-	-	-	0.96
Elemol	10.015	-	0.47	-	-
Sub total		22.28	12.23	18.57	18.23
Aldehyde					
beta.-Citronellal	5.202	-	-	1.15	-
Citronellal	5.230	-	0.55	-	2.07
Capri aldehyde	5.720	-	-	1.88	-
Capraldehyde	5.744	-	1.96	-	-
Beta -Citral	6.104	1.71	0.94	1.15	3.35
alpha.-Citral	6.385	2.74	2.17	1.65	2.11
Sub total		4.45	5.62	5.83	7.53
Ketone					
Pinocarveol	5.382	2.61	-	-	-
Carvone	5.805	-	-	0.45	-
Isopiperitenone	6.483	-	-	1.76	-
p-Mentha-1,8-dien-3-one	6.230	-	-	-	3.78
Sub total		2.61	0.0	2.21	3.78
Esther					
Geraniol acetate	7.518	0.91	-	-	-
Elaidic acid, methyl	16.593	-	-	0.74	-
Metholene 2216	14.700	-	-	1.04	-
Nerol acetate	6.383	-	-	-	3.97
Sub total		0.91	0.0	0.74	3.97
Sesquiterpene					
Caryophyllene	8.229	-	1.24	0.69	-
alpha.-Bergamotene	8.317	1.27	-	-	0.57
alpha.-Caryophyllene	8.708	-	0.38	0.36	-
alpha.-Cubebene	7.622	-	1.19	-	-
gamma.-Cadinene	7.777	-	0.75	-	-
Germacrene B	9.310	-	0.34	-	-
beta.-Bisabolene	9.353	1.53	-	-	3.91
beta.-Cadinene, (-)-	9.635	-	0.91	0.29	-

(+)-alpha-Longipinene	6.822	-	-	-	2.86
Beta.-Himachalene	6.958	-	-	-	0.60
Beta.-Santalene	7.669	-	-	-	1.63
Alpha.-Credence	7.742	-	-	-	2.05
Sub total		2.8	4.81	1.34	11.62
Oxide					
Caryophyllene oxide	10.602	1.94	-	-	1.38
Linalool oxide trans	4.569	0.81	-	-	-
cis-Linaloloxide	4.585	-	2.50	-	-
Limonene oxide, trans- Oxidized monoterpenes	5.075	-	-	0.49	-
Sub total		2.75	2.50	0.49	1.38
Others					
1,2-Cyclohexanediol, 1-methyl-4-(1-methylethenyl)-	7.136	1.77	-	-	-
3-t-Butyl-4-methoxyphenol methyl	8.061	3.64	-	1.20	-
alpha.-Methyl-.alpha.-[4-methyl-3-pentenyl]oxiranemethanol	4.413	1.35	1.35	-	-
Laurostearic acid	7.183	-	-	-	1.56
Myristic acid	8.732	-	-	-	1.51
Linoleic acid	9.658	-	-	-	1.06
Sub total		6.76	1.35	1.20	4.13
Grand Total		100%	100%	100%	100%

Table 2: Major components of Nigeria *citrus* species

S/No	Names of compounds	*Citrus aurantifolia* (% composition)	*Citrus paradise* (% composition)	*Citrus sinensis* (% composition)	*Citrus Limon* (% composition)
1.	Alpha-pinene	4.32	6.65	5.53	3.51
2.	Beta-pinene	23.65	15.81	13.56	-
3.	Limonene	29.22	50.37	48.68	42.21
4.	Beta-linalool	3.41	-	6.38	2.77
5.	Alpha-terpineol	2.79	1.83	2.11	3.62
6.	Beta-citral	1.71	0.94	1.15	3.35
7.	Alpha-citral	2.74	2.17	1.65	2.11

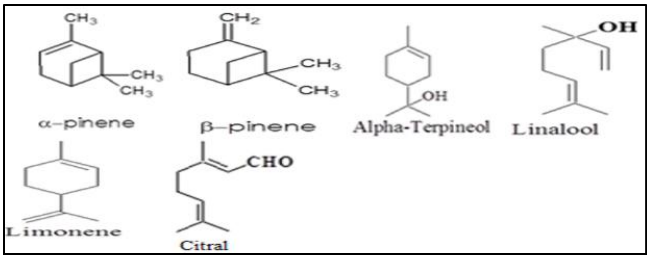

Fig 2: Structures of some major components of *citrus* peel oils from Nigeria

Discussion

The chemical composition of *Citrus* species studied was grouped into 8 (mono-terpenes, alcohols, aldehydes, ketones, esters, sesquiterpenes, oxides and other compounds). It was observed that Monoterpenes had higher percentage yield with *Citrus paradise* (72.83), *Citrus sinensis* (68.57), *Citrus aurantifolia* (57.19%), *Citrus Limon* (49.35). It was also observed that the four *Citrus* species had high alcohol with *Citrus aurantifolia* (22.28%), *Citrus sinensis* (18.57%), *Citrus Limon* (18.23%) and *Citrus paradise* (12.23%). The study had other compounds in smaller quantity for example, aldehydes with *Citrus Limon* (7.53%), *Citrus sinensis* (5.83%), *Citrus paradise* (5.62%) and *Citrus aurantifolia* (4.45%); Ketone with *Citrus Limon* (3.78%), *Citrus aurantifolia* (2.61%), *Citrus sinensis* (2.21%) and *Citrus paradisi* (0.0); Ester with *Citrus Limon* (3.97%), *Citrus aurantifolia* (0.91%), *Citrus paradise* (0.74%) and *C. sinensis* (); Sesquiterpenes with *Citrus aurantifolia* (2.8%), *Citrus paradisi* (4.81%), *Citrus sinensis* (1.34%), *Citrus Limon* (11.62%); Oxides with *Citrus aurantifolia* (2.75%), *Citrus paradisi* (2.50%), *Citrus sinensis* (0.49%), *Citrus Limon* (1.38%) and others compounds with *Citrus aurantifolia* (6.76%), *Citrus paradisi* (1.35%), *Citrus sinensis* (1.20%), *Citrus Limon* (4.13%). Limonene, alpha-pinene, alpha-terpineol, beta-citral and alpha-citral are present in all the species studied. *Citrus* essential oils (EOs) have been widely used for their bactericidal, virucidal, fungicidal, antiparasitical, insecticidal, medicinal and cosmetic proprieties. Also nowadays, they find important applications in pharmaceutical, sanitary, cosmetic, agricultural and food industries (Eristanna *et al*., 2013) [22]. In vitro physicochemical assays classify most of them as antioxidants. Monoterpenes are nonnutritive dietary components found in the essential oils of citrus fruits and other plants. A number of these dietary monoterpenes have antitumor activity (Crowell, 1999) [9]. Perillyl alcohol and d-Limonene also have chemotherapeutic activity against rodent mammary and pancreatic tumors (Crowell, 1999) [9]. Thus, monoterpenes would appear to act through multiple mechanisms in the chemoprevention and chemotherapy of cancer.

Conclusion

The isolation of the various components detected by the GCMS analysis can be used for the formulation of drugs used to treat ailments like cancer, ulcer, gastro esophageal reflux disorders, heartburns, anti-depressants and as flavoring agents in the manufacture of perfumes and cosmetics.

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