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Evaluation of the effect of drying on the chemical composition and antioxidant activity of the essential oil of peels from three species of citrus group

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Abstract

The effect of drying on the chemical composition and antioxidant activity of the essential oil obtained from peels of *Citrus. acida* (lime), *C. mandari*n (grape) and *C. reticulata* (tangerine) was examined. The result of the chemical composition as determined by gas chromatography/ mass spectrometry showed that monoterpene hydrocarbons, oxygenated monoterpene hydrocarbons and sesquiterpenes are the major terpenes found in the essential oils of both dried and fresh peels of the samples. All the dried samples had higher percentage of one terpene group or the other than their fresh counterpart. The percentage of oxygenated monoterpenes ranged between 7.03 – 21.71% with fresh lime peels having the highest percentage and fresh tangerine peels, the lowest percentage. All samples possessed antioxidant activity as evaluated by 1,1-diphenyl-2-picryhydrazyl (DPPH) radical method, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfunate) (ABTS*), Hydroxyl radical and ferric reducing property (FRAP). Dried tangerine peels showed the highest ABTS radical scavenging ability when compared with trolox. The results showed that drying had effect on the chemical composition as well as the antioxidant activity of the samples. Peels of these citrus species can be of importance in medicine, cosmetic and pharmaceutical industry due to their good antioxidant ability.

Introduction

Essential oils are natural aromatic oily liquids which are obtained from various plant parts such as flowers, buds, seeds, leaves, twings, bark, woods fruits and roots (Burt, 2007). They can also be defined as concentrated hydrophobic liquid containing volatile aroma compounds from plants (Wikipedia, 2013). Plants essential oils are rich sources of scents which are used in food preparation and preservation. Scientifically, these oils have been proved to have highly potent antimicrobial agents. They posses multiple antimicrobial i.e antibacterial, antifungal, anticancer, antiviral and antioxidant properties and these make them useful in food industry, pharmaceutical and cosmetics industry (Upadhyay *et al*., 2010).

Antioxidants are complexes found in our diet that act as protective shield for our bodies against certain diseases such as cardiac disease, arteritis etc. These agents are able to remove the deleterious effects of free radicals within our body by slowing or retarding the organic matter oxidation promoted by these free radicals (Mathur *et al*., 2011; Barja, 2014). Free radicals are produced in cells of living organisms when oxidative reactions occur (Fasola *et al.*, 2011), excessive production of these free radicals starts chain reaction and causes serious damage to cells; antioxidants however terminate these chain reactions by removing the excess free radical intermediates and inhibit other oxidation reactions by going through oxidation themselves (Fasola *et al*., 2011). Phenolic compounds, flavoniods and diterpenoids have been found to possess antioxidant activity and therefore are able to scavenge free radicals and inhibit lipid peroxidation by their reducing properties, acting as hydrogen or electron donating agents, singlet oxygen quenchers and metal chelators; thus, possessing a wide spectrum of biological effects including antioxidant and free radical scavenging ability (Pellati *et al*., 2004; Oh *et al*., 2013). Antioxidative action is one of the important physiological functions of foods because this helps to protect living organisms from oxidative damages resulting in the prevention of various diseases such as cancer, cardiovascular diseases and diabetes. Hence, considerable interest is being focused on development and evaluation of natural antioxidants and radical scavengers from plant material which are rich in polyphenolic compounds.

Food plants including fruits, vegetables and

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spices are primary sources of naturally occurring antioxidants for humans (Haddad and Dezashibi, 2007). Citrus species is one of the available fruits that are rich in polyphenols, flavonoids and terpenoids (Siddique *et al*., 2011; Oboh and Ademosun, 2012). The genus citrus (Rutaceae) comprises of trees, shrubs and herbs of various sizes and uses. They are most widespread arboreal plants in the world and represent one of the most important crops (Javed *et al*., 2013). The term citrus fruit includes different types of fruits and products (Mathur *et al.*, 2011). Although, oranges are the major fruit in the citrus fruits groups, accounting for about 70% of the citrus output, small citrus fruits such as tangerines, mandaris, clementine, lemon, lime and grapefruits are also included in the group.

Citrus fruits and their by- products are of increasing economic and medicinal value because of their numerous uses in pharmaceutical and cosmetic industries (Upadhyay *et al*., 2010). Peels of the citrus species normally referred to as waste correspond to about 20% of the raw processed fruits and this can be used as potential source of valuable by-product. They are good sources of valuable secondary plant metabolites and essential oils (Andrea *et al*., 1999; Ghasemi *et al*., 2009). They contain some antioxidants like flavanoids, polyphenols and terpenes (Marthur *et al*., 2011).

Numerous studies have analysed the essential oils and antioxidant activity of different species of citrus group but there are few works on the antioxidant activity of the essential oil of fresh and dried peels. Ayoola *et al.* (2008) evaluated the chemical constituents and the antimicrobial activity of the volatile oil of Citrus reticulata fruits from Southwest Nigeria. Javed *et al.* (2014) also investigated the antioxidant and antimicrobial potential of essential oil from five citrus species. Some studies have shown that, drying plant materials can exert significant effect on the chemical profile and biological attributes of essential oils derived from plants (Asekun *et al*., 2007a; Asekun *et al*., 2007b). This study aimed at analysing the essential oils from fresh and dried peels of three species of citrus groups available in Nigeria. We also examined the effect of drying on the chemical composition as well as the antioxidant activity of these oils using different antioxidant assays.

Materials and Methods

Collection of samples

The fruits of *Citrus acida* (lime), *C. mandarin* (grape) and *C. reticulata* (tangerine) were collected from local farms in Ekiti State, South West Nigeria. The fruits were washed and peeled. The peels were washed with distilled water and were divided into two parts; one part was dried before extraction while the other part was extracted immediately.

Extraction of essential oils

100 g of the grinded peel powder was subjected to hydrodistillation for 3h in hydro-Clevenger apparatus according to the method recommended by the European Pharmacopoeia (1997). The extracted oil sample was passed over anhydrous sodium sulphate and stored in sealed vials at 4^{o}C for further analysis.

Analysis of essential oil

The essential oil was analysed with gas chromatography/ mass spectrometry. The GCMS conditions of analysis are as follows: HP 6890 powered with HP ChemStation Rev. A09.01 [1206] software, split ratio: 20:1, carrier gas: Nitrogen, inlet temperature 250^{o}C, column type: HPINNOWax, Oven program: initial temperature at 60^{0}C, first Ramping at 12^{0}C/ min for 20 min, maintained for 2 min, second ramping at15^{o}C/min for 30 min maintained for 8 min, detector: free induction decay (FID), detector temperature: 320^{o}C

1,1- diphenyl -2-picrylhydrazyl free radical scavenging ability

The free radical scavenging ability of the essential oil against 1, 1-diphenyl -2- picrylhydrazyl (DPPH) free radical was evaluated as described by Gyanfin *et al.* (1999). Briefly an appropriate dilution of the extract (1 ml) was mixed with 1 ml of 0.4 mmol/L methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance was measured at 516 nm. The DPPH free radical scavenging ability was subsequently calculated with respect to the reference. The percentage DPPH radical-scavenging activity was calculated as:

$$(Abs_{ref} - Abs_{sam})/(Abs_{ref}) \times 100$$

Where:

Abs_{ref} – Absorbance of reference; Abs_{sam} – Absorbance of sample

The IC_{50} was calculated from the plot of absorbance and volume of extract

Fenton reaction (OH radical)

The ability of the extracts to prevent Fe^{2+}/H_2O_2-induced decomposition of deoxyribose was carried

out using the method of Halliwell and Gutteridge (1981). Briefly, appropriate dilution of the extracts were added to a reaction mixture containing 120 µl 20 mM deoxyribose, 400 µl 0.1 M phosphate buffer, 40 µl 20 mM hydrogen peroxide and 40 µl 500 µM $FeSO_4$, and the volume was made up to 800 µl with distilled water. The reaction mixture was incubated at 37°C for 30 min, and the reaction was then stopped by the addition of 0.5 ml of 2.8% trichloroacetic acid (TCA), this was followed by the addition of 0.4 ml of 0.6% thiobarbituric acid (TBA) solution. The test tubes were subsequently incubated in boiling water for 20 min. The absorbance was measured at 532 nm using spectrophotometer. The percentage (%) hydroxyl radical scavenging ability was subsequently calculated as:

$$(Abs_{ref} - Abs_{sam})/(Abs_{ref}) \times 100$$

Where:

Abs_{ref} – Absorbance of reference; Abs_{sam} – Absorbance of sample

The IC_{50} was calculated from the plot of absorbance and volume of extract.

2,2 – Azinobis (3- ethylbenzo-Thiazoline-6- sulfonate) Radical scavenging ability (ABTS)*

The 2,2 –azinobis (3- ethylbenzo-thiazoline -6 - sulfonate) (ABTS*) scavenging ability of the oils were determined according to the method described by Re *et al.* (1999). ABTS* was generated by reacting an ABTS* aqueous solution (7mmol/L) with $K_2S_2O_8$ (2.45 mmol/L, final concentration) in dark for 16h and by adjusting the Abs 734 to 0.700 with ethanol. An appropriate dilution (0.2 mL) of the extract was added to 2.0mL ABTS* solution and the absorbance was measured at 734nm after 15 min. The scavenging ability was compared with trolox as the standard and the percentage scavenging ability of the sample was calculated as:

$$(Abs_{ref} - Abs_{sam})/(Abs_{ref}) \times 100$$

Where:

Abs_{ref} – Absorbance of reference; Abs_{sam} – Absorbance of sample.

Determination of reducing property (FRAP)

The reducing property of the oil was determined as described by Bub *et al.* (2000) with minor modifications. The anti-oxidative activity of the oils was estimated by using the increase in absorbance caused by the generated ferrous ions. 100 µl aliquot of the essential oil was mixed with 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferric cyanide. The mixture was incubated at 50°C for 20 min and then 2.5 mL of 10% TCA was added. This mixture was centrifuged 650 rpm for 10 min. 5 mL of the mixture was mixed with 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric –reducing antioxidant property (FRAP) of the oil was compared with ascorbic acid.

Results and Discussion

The percentage oil yield of the samples ranged between 1.33 – 3.33%. Dried Tangerine had the highest percentage yield (3.33%) while fresh tangerine and dried lime had the lowest yield (1.33%). The percentage oil yield obtained from these samples is higher than in previously reported work. Mahmud *et al.* (2009) reported 0.31% for citrus acida peels; Javed *et al.* (2014) reported 0.45%, 0.37%, 0.33%, 0.30%, 0.28% for *C. paradise, C.sinensis* var Malta, *C. reticulate* Var Tangerine respectively. Yield of essential oil differs with individual plant species, seasonal variations, time of harvest and method of extraction. The differences in these results may be due to any of the factors mentioned above.

The chemical composition of the essential oils as presented in Table 1 showed limonene to be present at high percentage in all the samples. This is not unexpected because previous works had shown limonene to be the dominant terpene in the essential oils of citrus species (Kirbaslar *et al*., 2009; Tao *et al*., 2009; Yang *et al*., 2010; Kamal *et al*., 2013; Javed *et al*., 2014). Monoterpenes hydrocarbons, oxygenated monoterpenes hydrocarbons and sesquiterpene hydrocarbons were the major classes of terpenes present in the essential oils of the samples. The results of the total percentage of these classes of essential oil are presented in figure 1 while the breakdown of each class is shown in Table 1.

As shown in figure 1, monoterpenes had very high percentage in all the samples with the order: fresh tangerine > dried tangerine > dried grape > dried lime > fresh grape >fresh lime. Fresh lime had the highest percentage of oxygenated monoterpenes while fresh tangerine had the lowest percentage. Sesquiterpenes is present in small percentage in all the samples studied with fresh lime also having the highest percentage of 9.39% and fresh tangerine having the lowest percentage.

Diverse functional roles of terpenes have been critically studied and well-accepted. Some of them include as natural flavour additives for food,

Table 1. Essential oil of fresh and dried peels of lime, grape and tangerine

Essential Oils	F.L	D.L	F.G	D.G	F.T	D.T
Monoterpene HC						
Alpha Phenandrene	ND	0.09	0.06	0.10	0.15	0.10
Sabiene	ND	0.09	0.23	0.10	0.11	0.08
Alpha pinene	ND	6.31	1.36	1.96	1.86	1.35
Beta pinene	11.93	12.53	0.28	0.34	0.25	0.04
Limonene	33.14	53.79	60.63	64.02	75.59	67.34
Cis Ocimene	ND	0.08	0.06	0.09	0.13	0.09
Beta myrcene	1.06	2.43	8.11	8.21	11.49	12.75
Allo ocimene	ND	0.04	0.03	0.05	0.13	0.05
Gamma terpinene	4.82	0.31	0.76	0.38	0.53	0.56
Gejjerene	ND	0.08	0.05	0.09	0.13	0.08
Decanal	1.34	ND	ND	ND	ND	ND
Cyclo hexane- 1,1- dimethyl	2.79	ND	ND	ND	ND	ND
Cyclo pentane- 1- ethyl-2-methyl	2.09	ND	ND	ND	ND	ND
Octane	7.26	ND	ND	ND	ND	ND
Cyclohexane 1,4 dimethyl	1.21	ND	ND	ND	ND	ND
p- xylene	1.65	ND	ND	ND	ND	ND
p- cymene	1.61	ND	ND	ND	ND	ND
TOTAL	**68.90**	**75.75**	**71.57**	**75.34**	**90.37**	**82.44**
Oxygenated Monoterpene HC						
Alpha Thujene	ND	1.77	0.42	0.20	0.28	0.32
Decanal	ND	2.40	4.06	7.96	0.28	0.25
Fenchone	ND	0.09	0.06	0.10	0.14	0.10
Neral	ND	0.06	0.04	0.07	0.10	0.07
Geranial	ND	1.90	0.03	0.05	0.15	0.05
Isoartemisia	ND	0.04	0.03	0.04	0.06	0.04
1,8- cineole	ND	0.18	1.03	0.22	0.31	0.75
Geraniol	2.07	0.09	0.24	0.11	0.15	0.17
Nerol	ND	0.05	0.04	0.06	0.08	0.06
linalool	ND	2.75	2.83	3.27	1.23	1.68
Borneol	ND	0.23	0.68	0.27	0.38	0.44
Alpha Terpineol	3.36	3.96	4.12	4.71	1.50	2.37
Terpinen – 4- ol	2.19	0.42	0.71	0.51	0.71	0.65
Thymyl methyl Ether	ND	0.17	1.21	0.20	0.28	0.67
Ascaridole	ND	0.05	0.04	0.06	0.08	0.06
Linalyl Acetate	ND	0.13	0.57	0.16	0.22	0.27
Ethyhl Cinnamate	ND	0.19	0.61	0.22	0.32	0.38
Borneol Acetate	ND	0.18	0.84	0.21	0.30	0.50
Neryl Acetate	ND	0.04	0.03	0.05	0.06	0.04
Geranyl Acetate	ND	5.05	1.30	0.94	0.25	0.35
Acetyl Eugenol	ND	0.03	0.02	0.04	0.05	0.03
Benzyl Benzoate	ND	0.06	0.04	0.07	0.10	0.07
1,6 octadien 3 –ol, 3,7- dimethyl	2.13	ND	ND	ND	ND	ND
2,6- Octadien -1- ol, 3,7- dimethyl	1.49	ND	ND	ND	ND	ND
Trans Citral	6.13	ND	ND	ND	ND	ND
Cis Citral	4.34	ND	ND	ND	ND	ND
TOTAL	**21.71**	**19.84**	**18.95**	**19.52**	**7.03**	**9.32**
Sesquiterpene						
Beta Cryophyllene	1.50	2.82	4.50	3.35	1.80	6.41
Trans Alpha Bergamotene	2.03	0.07	0.05	0.09	0.12	0.08
Gamma Cardinene	ND	0.05	0.04	0.06	0.08	0.06
Bicyclogermacrene	ND	0.11	1.55	0.13	0.16	0.59
Germacrene	ND	1.20	2.99	1.42	0.28	0.88
Elemicin	ND	0.02	0.01	0.02	0.03	0.02
Alpha Humulene	ND	0.09	0.20	0.10	0.15	0.15
Beta Elemene	ND	0.02	0.02	0.03	0.04	0.02
Cis Caryophyllene	4.43	ND	ND	ND	ND	ND
2- methyl-3,13-octadecadienol cycldodecyne	1.43	ND	ND	ND	ND	ND
TOTAL	**9.39**	**4.38**	**9.36**	**5.20**	**2.66**	**8.21**

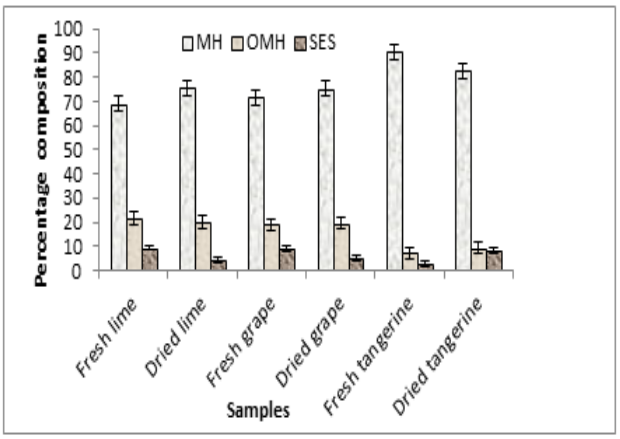
HC- Hydrocarbon, ND – Not Detected, F.T- Fresh Tangerine, D.T- Dried Tangerine, F.L – Fresh Lime, D.L – Dried Lime, F.G – Fresh Grape, D.G – Dried Grape

fragrances in perfumery and in traditional and alternate medicines as aromatherapy, they also have ability to prevent and treat cancer. Other important therapeutic uses of terpenes include antimicrobial, antifungal, antiviral, antihyperglycemic, anti-inflammatory, antioxidants, antiparasitic, immunomodulatory, and as skin permeation enhancer (Brahmkshatriya and Brahmkshatriya, 2013).

Citral was found only in fresh lime while dried tangerine had highest percentage of beta myrcene. Figure 2 showed the effect of drying on the components of the essential oils. As shown in Figure 2, all the dried samples had relatively high percentage of one terpene group than their fresh counterpart (although it did not follow the same trend). This showed that drying had little effect on some of the chemical constituents of the essential oil and thus resulting in a little variation in the chemical constituents. Hence, given their high perishability, the samples can be preserved by drying without losing their biologic potentials and health benefits being explored by the pharmaceutical and cosmetic industries (Kourounakis *et al*., 1999).

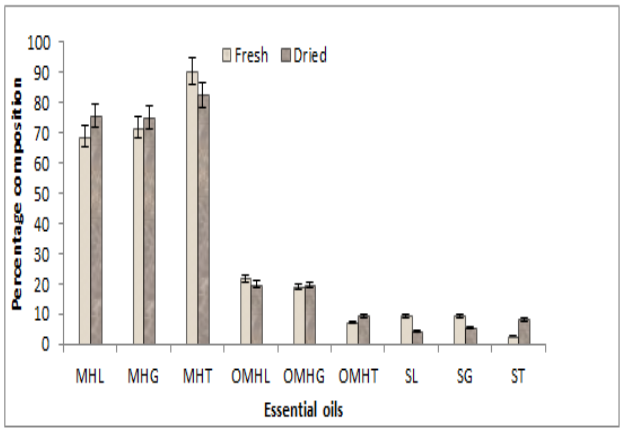
Antioxidant activity

Antioxidation is a complex process usually occurring through several mechanisms (Aruoma, 2003) therefore, evaluation of antioxidant activity of extracts from natural samples are usually done by more than one method. In this study, we used four methods (DPPH, OH radical, FRAP and ABTS) as mentioned above. The result of the IC_{50} calculated using DPPH assay showed that the IC_{50} of the oils ranged between 101.10 – 387.60 g/ml; with fresh lime having the lowest value of 101.10 g/ml and fresh grape having the highest value of 387.60 g/ml. This showed that fresh lime peels had the highest DPPH radical scavenging ability followed by dried grape (IC_{50}= 142. 45 g/ml). The good DPPH radical scavenging activity of fresh lime may be attributed to the high percentage of oxygenated monoterpenes hydrocarbon present in the essential oil of the sample. Oxygenated monoterpenes have been found to have good antioxidant activity because they undergo



MH- Monoterpene hydrocarbons, OMH- Oxygenated monoterpene hydrocarbons, SES- Sesquiterpenes

Figure1. Percentage composition of essential oil of fresh and dried samples of lime, grape and tangerine



MHL, MHG, MHT – monoterpenes hydrocarbons of lime, grape & tangerine respectively;

OMHL, OMHG, OMHT – oxygenated monoterpene hydrocarbon of lime, grape & tangerine respectively;

SL, SG, ST – sesquiterpenes of lime, grape & tangerine respectively

Figure 2. Effect of drying on the components of the essential oils

antioxidation, characterized by very fast termination process thereby reducing overall rate of oxidation (Amorati, 2013). Nerol, perillyl alcohol, geraniol and cis- verbenol have also been found to have relatively high antioxidant activity (Riachi *et al*., 2015). Fresh lime had the highest percentage of geraniol coupled with citral and thus their reaction with DPPH radical. DPPH radical is able to react with reactive oxygen species and also with unsaturated hydrocarbons by abstracting an H – atom from C-H bonds with a sufficient low bond dissociation enthalpy (Riachi *et al*., 2015) thus, discoloration of DPPH in the presence of fresh lime could be attributed to these essential oil and some other unsaturated terpenes present in it.

Fresh grape showed the highest OH- radical scavenging activity (IC_{50} = 102.04 g/ml) followed

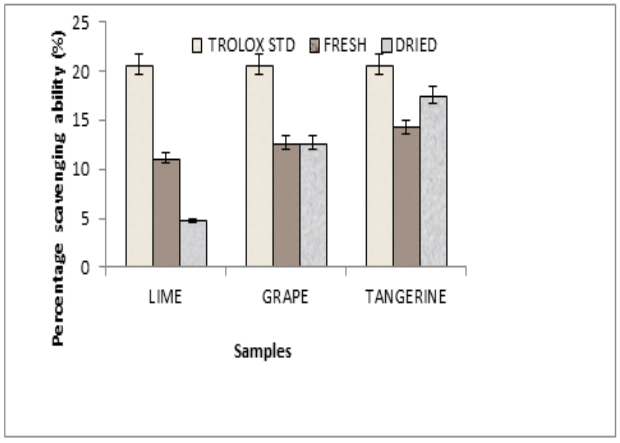


Figure 3. Percentage scavenging ability of samples using ABTS* radical

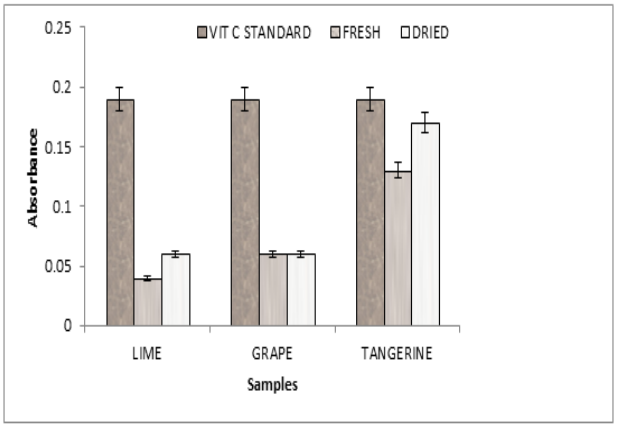


Figure 4. Radical scavenging ability using FRAP assay

by dried tangerine and dried grape (IC_{50}= 105.04 g/ml, 107.30 g/ml respectively). This also could be attributed to the high percentage of 1,8- cineole and linalool in these oils. These oxygenated monoterpenes hydrocarbons have been found to be highly reactive and could also inhibit hexanal oxidation (Lee *et al*., 2005). The result of the antioxidant activity of these oils is comparable with the antioxidant activity of essential oil of flowers of Azidrachia indica plant reported by El Hawary *et al.* (2013) where he compared the antioxidant activity of leaves and flowers. He found that the flowers had the highest percentage of oxygenated monoterpenes compound and thus the highest DPPH radical scavenging ability.

The results of the ABTS and FRAP assays are presented in Figures 3& 4 respectively. The result showed that tangerine essential oil had good antioxidant activity in the two assays followed by grape in ABTS assay. This could be attributed to the presence of high quantity of beta myrcene and limonene in both samples. Yang *et al.* (2010) reported that limonene is a major constituent of citrus peel oils having antioxidant potential equivalent to that of a strong antioxidant.

Dried peels of tangerine and grape showed good antioxidant activity with ABTS assay than their fresh peels while dried peels of lime showed good antioxidant activity with FRAP assay. This could be attributed to some of the component of the essential oils that have been increased as result of drying as shown in Figure 2 and Table 1. This further stressed that drying had effect on the chemical constituents and the antioxidant activity of peels of some citrus species.

All samples showed good antioxidant activity and therefore they can find application in pharmaceutical industry and also in medicine because they have shown ability to absorb and neutralize free radicals which when present in high percentage in a particular organism tend to exceed the antioxidant capacity of the organism thereby leading to a variety of pathophysiological processes such as inflammation, diabetes, genotoxicity and cancer (Kourounakis *et al.*, 1999).The scavenging action of these essential oils could also have a practical application in aesthetic medicine (branch of medicine focused on satisfying the aesthetic desires and goals of patients) and for treating human skin oxidative damage.

Conclusion

This study highlights the industrial, pharmaceutical and medical potentials of the samples studied. Ordinarily, these samples would have been regarded as waste materials. Their essential oils had very high percentage of monoterpenes hydrocarbons and relatively high percentage of oxygenated monoterpenes hydrocarbons. All the dried samples had higher percentage of either monoterpene hydrocarbon, oxygenated monoterpene hydrocarbon or sesquiterepene hydrocarbon compared to their fresh samples and this showed that drying had effect on the chemical composition of essential oils in the peels. The result of the antioxidant activity of the oils also showed that drying had effect on the antioxidant activity of the oils as dried tangerine showed good antioxidant activity in some of the antioxidant assay. All samples had good radical scavenging ability and this showed that they can be of importance in medicine, pharmaceutical and cosmetic industries instead of discarding them as mere waste materials.

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References

Amorati, R., Foti, M.C.and Valgimigli L. 2013. Antioxidant activity of essential oils. Journal of Agricultural and Food Chemistry 61(46):10835-10847.

Andrea, V., Nadia, N., Teresa, R.M. and Andrea, A. 2003. Analysis of some Italian lemon liquors (limocello). Journal of Agricultural and Food Chemistry 51(17):4978 – 4983.

Aruoma, O. 2003. Methodological consideration for characterizing potential antioxidant actions of bioactive components in plant foods. Mutation Research 523-534: 9-20.

Asekun, O.T., Grierso, D.S. and Afolayan, A. J. 2007a. Effect of drying methods on the quality and quantity of the essential oil of *Mantha longifolia* L subsp. *Capansis*. Journal of Essential oil Research 101(3):995-998.

Asekun, O.T., Grierson, D.S. and Afolayan, A.J. 2007b. Characterization of essential oils from *Helichrysum odoratissimum* using different drying methods. Journal of Applied Science 7: 1005-1008.

Ayoola, G.A., Johnson, O.O., Adelowotan, T., Aibinu, I.E., Adenipekun, E., Adepoju –Bello, A.A., Coker, H.A.B. and Odigbemi, T.O. 2008. Evaluation of the chemical constituents and the antimicrobial activity of the volatile oil of citrus reticulata fruit (Tangerine fruit peel). African Journal of Biotechnology 7(3): 2227-2231.

Barja, G. 2014. The mitochondrial free radical theory of aging. Progress in Molecular Biology and Translational Science 127:1-27.

Brahmkshatriya, P.P. and Brahmkshatriya, P.S. 2013. Terpenes: Chemistry, Biological Role and Therapeutic Applications Natural Products 2665-2691.

Bub, A., Watzl, B., Abrahamse, L., Delincee, H., Adam, S. and Wever, J., Federation of European Biochemical Societies Letters 2000. Moderate intervention with carotenoid-rich vegetable products reduce lipid peroxidation in men. Journal of Nutrition 130(9): 2200–2206.

Burt, S.A. 2007. Antibactrerial activity of essential oils: potential applications. In Drukkerij B.V. and Ridder .K. (Eds). Food, p. 3. Netherlands: Ridder print offset.

El Adaway, T.A., Rehman, E.H., El- Bedaway, A.A. and Gafar, A.M. 1999. Properties of some citrus seeds part 3. Evaluation as new source of potential oil. Food/ Nahrung 43(6): 385-391.

El Hawary, S.S., El – Tantawy, M.E., Rabel, M.A. and Badr, W.K. 2013. Chemical composition and biological activities of essential oils of *Azadirachta indica* A. Juss. International Journal of Applied Research in Natural Product 6(4):33-42.

European Pharmacopoeia. 1997. Strasbourg: Council of European 3rd ed. European Pharmacopoeia.

Fasola, T.R., Oloyode, G.K. and Aponjolosun, B.S. 2011. Chemical composition, toxicity and antioxidative activities of essential oils of stem bark of Nigerian species of Guava (*Psidium guajava* Linn). Experimental and Clinical Sciences 10: 34- 43.

Ghasemi, K., Ghasemi, Y. and Ebrahim Zadeh, M.A. 2009. Antioxidant Activity, phenols, flavonoid contents of 13 citrus species peels and tissues. Parkistan Journal of Pharmaceutical Sciences 22(3):271-281.

Gyamfin, A., Yonamine, M. and Aniya, Y. 1999. Free radical scavenging action of medicinal herbs from Ghana: *Thoonningia sanquinea* on experimentally – induced liver injuries. General Pharmacology 32 (6): 661-667.

Haddad, K.M.H. and Dezashibi, Z. 2007. Phenolic compounds and antioxidant activity of Henna leaves extract *(Lawsonia inermus)*. World Journal of Diary and Food Sciences 2(1):38- 41.

Halliwell, B.and Gutteridge, J. M.C. 1981. Formation of a thiobarbituric- acid reactive substance from deoxyribose in the presence of iron salts. Federation of European Biochemical Societies Letters 128(2): 347-352.

Javed, S., Ahmad, R., Shahzad, K., Nawaz, S., Saeed, S. and Yasar, S. 2013. Chemical constituents antimicrobial and antioxidant activity of essential oil of citrus limetta var. mitha (sweetling peel) in Pakistan. African Journal of Microbiology Research 7(24): 3071 – 3077.

Javed, S., Javaid, A., Nawaz, S., Saeed, M.K., Mahmood, Z., Siddiqui, S. Z. and Ahmad, R. 2014. Phytochemistry, GC-MS analysis, antioxidant and antimicrobial potential of essential oil from five citrus species. Journal of Agricultural Science 6(3):202 - 208.

Kamal, G. M., Ashraf, M. Y., Hussain, A. I., Shahzadi, A. and Chughtai, M.I. 2013. Antioxidant potential of peel essential oils of three Pakistan *Citrus* species: *Citrus reticulata, Citrus sinensis* and *Citrus paradisii.* Pakistan Journal of Botany 45(4): 1449-1454.

Kirbaslar, F.G., Tavman, A., Dulger, B. and Turker, G. 2009. Antibacterial activity of Turkish Citrus Peel oils. Pakistan Journal of Botany 41(6): 3207-3212.

Kourounakis, A.P., Galanakis, D. and Tsiakirzis, K. 1999. Synthesis and pharmacological evaluation of novel derivatives of anti –inflammatory drugs with increased antioxidant and anti inflammatory activities. Drug Research 47 (1): 9-16.

Lee, S.J., Umano, K., Shibamoto, T. and Lee, K.G. 2005 . Identification of volatile components in basil (*Ocimum basilicum* L) and thyme leaves (*Thymus vulgaris* L) and their antioxidant properties. Food Chemistry 91: 131-137.

Mahmud, S., Saleem, M., Siddique, S., Ahmed, R., Khanum, R. and Perveen, Z. 2009. Volatile components, antioxidant and antimicrobial activity of *Citrus acida* var. sour lime peel oil. Journal of Saudi Chemical Society 13(2): 195-198.

Mathur, A., Verna, S.K., Purohit, R., Gupta, V., Dua, V.K., Prasad, G.B. and Mathur, D. 2011. Evaluation of *in vitro* antimicrobial and antioxidant activities of peel and pulp of some citrus fruits. IJPI'S Journal of Biotechnology and Biotherapeutic 1(2):1-17.

Oboh, G.and Ademosun, A.O. 2012. Characterization of the antioxidant properties of phenolic extracts from some citrus peels. Journal of Food Science and Technology 49(6): 729 – 736.

Oh, J., Jo, H., Cho, A.R., Kim, S.J. and Han, J. 2013. Antioxidant and antimicrobial activities of various leafy herbal teas. Food Control 31(2): 403-409.

Pellati, F., Benvenuti, S., Magro, L., Melogari, M. and Soragni, F. 2004. Analysis of phenolic compounds and radical scavenging activity of *Echinacea* spp. Journal of Pharmaceutical and Biomedical Analysis 35: 289-301.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M.and Rice –Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Radical Biology and Medicine 26(9 -10): 1231 – 1237.

Riachi, L.G. and De Maria, C.A.B. 2015. Peppermint antioxidants revisited. Food Chemistry 176: 72-81.

Siddique, S., Shafique, Z., Parveen, S.and Khan Khanum, R. 2011. Volatile components, antioxidant and antimicrobial activity of *Citrus Aurantiumvar* bitter orange peel oil. Pharmacology online 2: 499-507.

Tao, N., Liu, Y.and Zhang, M. 2009. Chemical composition and antimicrobial activities of essential oil from the peel of bingtang sweet orange (*Citrus sinensis* Osbeck). International Journal of Food Science and Technology 44(7): 1281–1285.

Upadhyay, R.K., Divividi, P. and Ahmad, S. 2010. Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. Asian Journal of Medical Sciences 2(3):152- 158.

Wikipedia the free Encyclopedia 2013. Essential oil. Retrieved on April 24, 2014 from Website: *http: en.wikipedia.org/wiki/essential_ oil*

Yang, S.A., Jeon, S. K., Lee, E. J, Shim, C. H. and Lee, I. S. 2010. Comparative study of the chemical composition and antioxidant activity of six essential oils and their components. Natural Product Research 24(2):140-151.