Tanzania Journal of Science 46(2): 419-429, 2020
ISSN 0856-1761, e-ISSN 2507-7961

Extraction, Derivatization, Characterization and Antifungal Investigation of Limonene from *Citrus sinensis* Peels

Ita E Uwidia*, Bodunde J Owolabi and Richard C Okafor
Department of Chemistry, Faculty of Physical Sciences, University of Benin, P.M.B. 1154, Benin City, Edo State, Nigeria.
**Corresponding author: e-mail: uwidiaie@yahoo.com*
E-mail co-authors: owolabibodunde@yahoo.com (Owolabi); blazedoperichie@gmail.com (Okafor)

Received 6 April 2020, Revised 31 May 2020, Accepted 4 June 2020, Published June 2020

Abstract

Limonene, a cyclic monoterpene is the main component in citrus oils. Oranges (*Citrus sinensis*) were sourced from a local market, peeled and the peels collected for this study. The orange peels were distilled using steam distillation to obtain the oil. The oil obtained was then distilled to obtain a pure colourless form of limonene (95%). The limonene (2 ml) was dissolved in dry chloroform (25 ml) and freshly distilled hydroiodic acid (57%; boiling point 127 °C) to give the derivative iodo-limonene (1.4 g; 30% yield). Another reaction of epoxidation of limonene was carried out by reacting limonene with hydrogen peroxide and acetic acid to give limonene epoxide (2.3 g; 36% yield). FT–IR analyses and GC-MS analyses were carried out to confirm the properties of the limonene. Antifungal tests on limonene against two fungi: *Trichoderma harzianum (T. harzianum)* and *Marcophomina phaseolina (M. phaseolina)* were carried out using poisoned food technique. 9 ml of potato dextrose agar (PDA) and 1 ml of limonene extract were used on both fungi. Petri dishes of agar without limonene served as control. Biological activities of the species were: *T. harzianum* culture 1 day 1-4: 0.00 mm ≡ no growth, day 5:1.5 mm → slight growth; *T. harzianum* culture 2 day 1–5: 0.00 mm ≡ no growth; *M. phaseolina* culture 1 days 1–4: 0.00 mm ≡ no growth, day 5:1.5 mm; *M. phaseolina* culture 2 days 1–3: 0.00 mm ≡ no growth, day 4: 1.50 mm → slight growth, day 5: 2.10 mm progressive growth. Results showed that both fungi were active in all media. Percentage inhibition of limonene against both fungal species using 1 ml of limonene was 95% for *T. harzianum* and 89% for *M. phaseolina.*

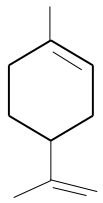
Keywords: Limonene, Extraction, Characterization, Antifungal, Epoxide.

Introduction

Limonene, one of the most abundant naturally occurring components of cannabis essential oils, is a plant product of terpenes. It is present in concentrations as high as 16% of essential oil fractions. Limonene is a clear, colourless hydrocarbon classified as a cyclic monoterpene, and is the major component in oil of citrus fruit peels most widely distributed in nature. It occurs naturally in orange peels, lemon, dill, mint, cumin and neroli. Limonene occurs in two optically active forms, (S–(–)–limonene and R–(+)-limonene). It also has two isomeric forms (*D*–isomer and *L*–isomer). *D*–limonene, a monocyclic terpene, occurs more commonly in nature. It is the fragrance of oranges and is used as a flavouring agent in food manufacturing (Ludwiczuk et al. 2017). Studies have shown that *D*–limonene is toxic to animals such as dogs (680 g/kg dosage) and cats. Also it has insecticidal properties and acts primarily as a desiccant for all life stages

of insects such as flea, ticks, etc. Therefore, it is a component of some products made from citrus oils such as soaps, shampoos, cleansers, solvents, fragrances, and insecticides (Plumlee 2013). The less common *L*-isomer is found in mint oils and has a piney, turpentine-like odour. Anti-tumour activities of the compound in several animal tumour models and *in vitro* experiments have been reported (Amanzadeh et al. 2006, Barceloux 2008).

The structure of limonene is as shown in Figure 1. Limonene, a cyclohex-1-ene substituted by a methyl group at position 1 and an isopropenyl group at position 4, has a molecular formula of $C_{10}H_{16}$. Therefore, the name of the compound is 1-methyl-4-(isopropenyl) cyclohex-1-ene. It has an average mass of 136.23404 g/mol.



Limonene

Figure 1: Structure of limonene

There is convincing epidemiological evidence that the consumption of orange fruit is beneficial to health and contributes to the prevention of degenerative processes, particularly lowering incidence of degenerative process, cardio and cerebro-vascular diseases. The protection that the orange fruit provides against these diseases has been attributed to the various antioxidant phytonutrients contained in citrus species (Falk-Filipsson et al. 1998, Obidi et al. 2013).

In some parts of the world, sweet oranges (*Citrus sinensis*) are cheaply available, and can serve as major sources of vitamins in diets. Orange fruit and its juice have several beneficial, nutritive and health properties. They are rich in vitamins, especially ascorbic and folic acids. Over the last decades, many other applications and medicinal benefits of orange fruits have been discovered besides their anti-scurvy properties (Ezejiofor et al. 2011). In Nigeria, orange peels are wastes thrown into the rubbish heaps to rot or be burnt. The aim of this study was therefore to extract and characterise limonene oil, evaluate its antifungal potentials (using the fungi *Trichoderma harzianum* and *Macrophomina phaseolina),* prepare limonene derivatives (iodo-limonene and epoxylimonene) and compare their organoleptic properties with the limonene oil.

Materials and Methods

Source of materials

The orange peels used for analysis were obtained from oranges sold at Uselu market, an indigenous market in Egor Local Government Area, Benin City Edo State, Nigeria in May, 2018.

Fungi isolates

The two fungi used for antimicrobial investigations (i.e., *Trichoderma harzianum* and *Macrophomina phaseolina)* were procured from Blue Bird Laboratory, Isiehor, Benin City, Edo State, Nigeria. *Trichoderma harzianum* is a genus of fungi in the family of Hypocreaceae. It is present in all soils where they are the most prevalent culturable fungi. The genus *Trichoderma harzanium* has gained novel biological properties and technological applications. It is an efficient biocontrol agent that can act against target organisms in diverse ways. Strains of *Trichoderma* are capable of producing antifungal antbiotics and extra cellular enzymes.

Morphological characteristics of *Trichoderma harzianum* show that it is a rapidly growing fungal strain on PDA plate (within a 42 hour whole PDA plate covered with mycelium) and form white cottony mycelium with green conidial production; with the conidial production denser at the centre than towards the margins (Koshle et al. 2016).

Marcophomina phaseolina is a botryosphaeriaceae plant pathogen fungus that

causes damping off, seedling blight, collar rot, stem rot, charcoal rot, basal stem rot and rot stem in many species of plants (Javaid and Rehman 2011). *Marcophomina phaseolina* grows on the roots surface, and germ tubes on the end of the microsclerotia form appresoria that penetrate the host epidermal cell wall.

Extraction of oil from orange peels

The fresh orange peels were obtained from oranges and washed with clean water. The peels were then cut into bits and put into a 1 litre round bottomed flask. The flask was filled with water to about ¾ capacity and the peels were allowed to soak overnight to allow (for) enough saturation in the water. Thereafter, the apparatus was set up for steam distillation (Ezejiofor et al. 2011). Heat was supplied through a hot plate. The oil distilled with water at 100 ºC. The resulting distillate was transferred into a separating funnel and allowed to stand for complete separation. The upper oil layer was removed and stored and the lower layer was run off. This procedure was repeated three times to obtain enough oil for the derivatives. The total oil obtained was then washed with water, dried over $MgSO_4$ overnight, filtered and purified by distillation at 176 ºC to obtain 95% limonene.

Synthesis of iodo - limonene from limonene oil

0.5 ml of the limonene was measured out into a beaker and 1 ml of hydroiodic acid was added and stirred together. Also, 0.5 ml of chloroform was added to the mixture above. After a while, the chloroform mixture was then heated gently. A capillary tube was used to spot the compound on a Thin Layer Chromatography (TLC) paper. The spotted TLC paper was placed in a beaker containing 2 ml of chloroform with the spots directly inserted. There was an observed flow of chloroform through the paper. When the flow had barely reached the near end of the paper, the paper was removed, air-dried and transferred to an iodine chamber. The spotting movement was then observed and the result recorded. Reaction of limonene with hydroiodic acid to give iodolimonene (1.4 g; 30% yield) is as shown in Figure 2.

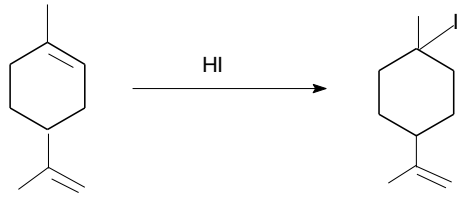


Figure 2: Reaction of limonene with hydroiodic acid to give iodolimonene.

Epoxidation of limonene

Epoxidation was carried out following basic epoxidation methods with slight modification. Acetic acid (60 ml) was added to 30 ml of hydrogen peroxide and mixed. 5 ml of the limonene was then added and the mixture was heated at 70 – 80 °C for 3 hrs. The reaction was monitored with spotting on TLC until the starting material had disappeared (3 hrs). The volume was reduced by distillation under reduced pressure. The remaining liquor was treated with saturated Na_2CO_3. After the initial effervescence had subsided, the mixture was cooled and extracted with chloroform, dried over $MgSO_4$ solvent to give a colourless liquid (yield 2.3 g; 36%). Retention factor, Rf: 0.45 ($CHCl_3/C_4H_8O_2$) (Katsuki and Sharpless 1980, Ikhuoria and Gbenga 2007).

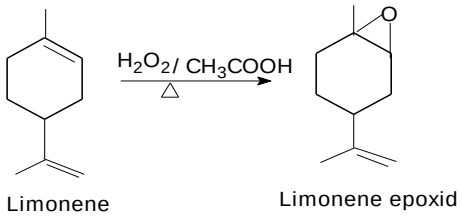


Figure 3: Epoxidation of limonene.

Antifungal investigations

The antifungal investigation was determined by poisoned food technique as described by Mohana and Raveesha (2007) and Đurović-Pejčev et al. (2014) with slight modifications.

First, the petri dishes were sterilized overnight in a sterilizing chamber at 121 ºC. 9 ml each of the potato dextrose agar (PDA) was measured and poured into eight sterilized plates and then allowed to cool and solidify. Out of the eight petri dishes, four were used to analyse each fungi. After complete solidification of the medium, 1 ml of limonene (quantitative estimate chosen) was introduced into two of the petri dishes and mixed thoroughly with the PDA. The remaining two dishes containing PDA without limonene were used as control. Using a sterilized borer, the fungal spore (0.5 mm) was picked and placed in the four dishes. All four dishes were incubated at 25 ºC and the growth was monitored for five days. The above procedure using the first four petri dishes was for the analyses of *Trichoderma harzianum*. This same procedure was repeated for the analyses of *Macrophomina phaseolina* using the remaining four petri dishes. The fungal growth was recorded and the percentage inhibition was calculated using the formula:

$$\text{Percentage inhibition} = \frac{\text{C -T}}{\text{C}} \times \frac{100}{1}$$

where: C = average increase in fungal growth in control plate and;
T = average increase in fungal growth in treatment plate.

Results and Discussion

Results obtained from the analyses are as shown in Tables 1- 4 and Figures 4, 5, 7 and 8. Identifications were carried out in terms of state, odour, texture and colour. Organoleptic properties of limonene compared with the oil extract from the analyses are as shown in Table 1. Also Table 2 shows the organoleptic properties of the limonene derivatives prepared and yields obtained. Table 1 shows organoleptic properties of limonene obtained from the analysis compared with theoretical properties.

Table 1: Comparative analysis of organoleptic properties of limonene obtained from extraction with theoretical properties

Properties	Limonene	Limonene oil (extract) obtained
State	Liquid	Liquid
Odour	Fruity lemon smell	Fruity lemon smell
Texture	Oily	Oily
Colour	Slight golden yellow –colourless	Colourless

Table 2: Organoleptic properties of limonene derivatives prepared

Properties	Iodo-limonene	Epoxylimonene
State	Liquid	Liquid
Odour	Characteristic odour	Metallic smell
Texture	Oily	Oily
Colour	Slight purple	Colourless, clear liquid
Yield	1.4 g; 30%	2.3 g; 36%

The FT-IR results of the limonene oil are as presented in Figure 4. This analysis was carried out to obtain the different functional groups in the sample. Limonene showed a characteristic peak at 3082 cm^{-1} which shows C-H stretch with sp^2 hybridization. Peak at 2836.5 cm^{-1}- 2967 cm^{-1} shows a C-H stretch with sp^3 hybridization. Peak 1643.8 cm^{-1} shows the presence of C=C of alkenes. The peak at 1375.4 cm^{-1} and 1438.8 cm^{-1} show CH_2 and CH_3 bonds in the compound. Also peaks at 887.1 cm^{-1} and 797.7 cm^{-1} show mono-substituted and tri-substituted alkenes, respectively.

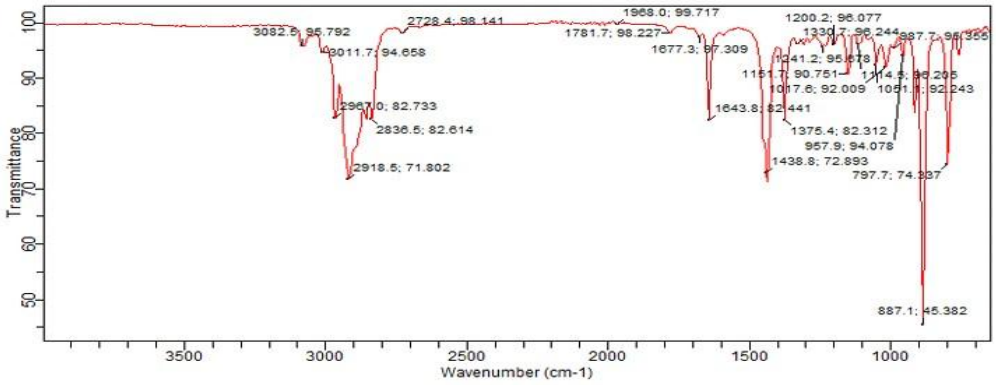


Figure 4: FT-IR spectrum of limonene.

Figure 5 shows the mass spectrum result for the analysis of limonene. From the spectrum, it is seen that the GC-MS analysis of limonene gave a maximum peak at m/z 138, which is in correlation with the theoretical molar mass of limonene at 136 g/mol. Therefore, the extracted limonene has a molar mass of 138 g/mol which helps to confirm that the structure analysed was limonene. The slight difference (+2) in the molar mass should be due to fragmentation. Limonene undergoes Retro-Diels-Alders reaction at the mass spectrometry fragmentation to give two molecules of isoprene (Figure 6). All the molecules involved except limonene are short lived. The transition state is recorded as M + 2 (Silverstein et al. 1974).

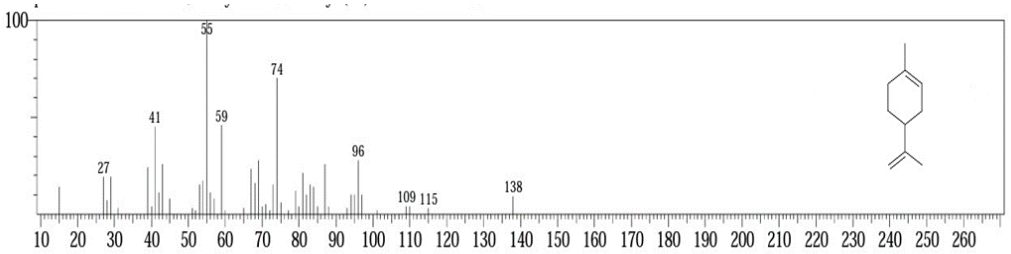
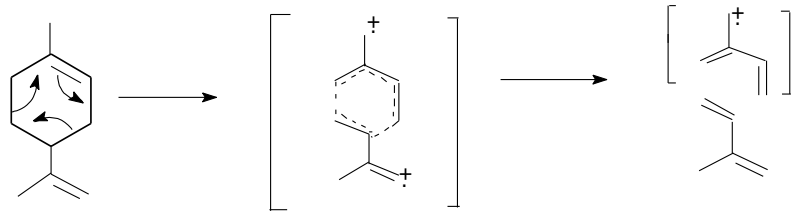


Figure 5: GC-MS spectrum of limonene.



M+2 138

Figure 6: Retro-Diels-Alders fragmentation in limonene.

Results of antifungal analysis

Results presented in Table 3 show that the *Trichoderma harzianum* reached full growth at day 4 of incubation in control 1 and control 2 (cultures without treatment with limonene). However for treated 1 (first culture treated with limonene) no growth was observed from day 1 to day 4 but from day 5 growth of 1.50 mm was observed. Also for treated 2 (second culture treated with limonene) no growth was observed from day 1 to day 5 of incubation. This result showed that reasonable growth inhibition against *Trichoderma harzianum* was achieved in both cultures treated with limonene. The percentage inhibition of limonene was 95%. The growth and inhibition results of the *Trichoderma harzianum* are as presented in Figure 7.

Table 3: Antifungal test results using 1 ml of limonene on *Trichoderma harzianum*

Days	Control 1	Control 2	Treated 1	Treated 2
	Diameter of zone of growth in mm			
Day 1	5.30	4.50	0.00	0.00
Day 2	7.90	7.10	0.00	0.00
Day 3	10.50	9.90	0.00	0.00
Day 4	17.00	17.00	0.00	0.00
Day 5	17.00	17.00	1.50	0.00

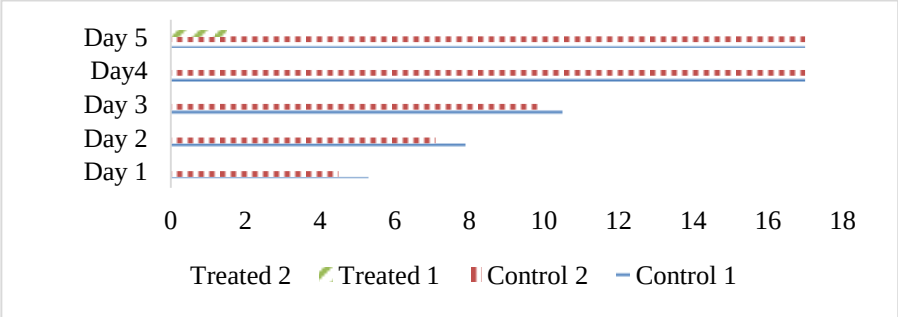


Figure 7: Growth and zone of inhibition of *Trichoderma harzianum.*

Also in Table 4, *Macrophomina phaseolina* reached full growth at day 5 of incubation in controls 1 and 2 which were cultures not treated with limonene. For treated 1 (first culture treated with limonene) no growth was observed from day 1 to day 4 but from day 5 growth of 1.50 mm was observed. For treated 2 (second culture treated with limonene) no growth was also observed from day 1 to day 3 of incubation. From day 4, growth of 1.5 mm was observed and at day 5 the growth was 2.10 mm. This also indicated that reasonable growth inhibition of *Macrophomina phaseolina* was achieved in both cultures treated with limonene. The percentage inhibition of limonene was 89%. The growth and inhibition results of the *Macrophomina phaseolina* are as presented in Figure 8.

Table 4: Antifungal test results using 1 ml of limonene on *Macrophomina phaseolina*

Days	Control 1	Control 2	Treated 1	Treated 2
	Diameter of zone of growth in mm			
Day 1	7.20	7.90	0.00	0.00
Day 2	8.50	8.50	0.00	0.00
Day 3	11.80	12.00	0.00	0.00
Day 4	13.20	14.30	0.00	1.50
Day 5	17.00	17.00	1.50	2.10

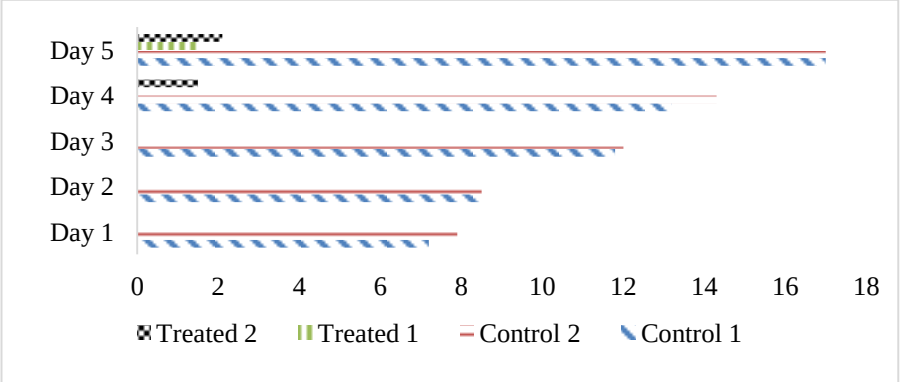


Figure 8: Growth and zone of inhibition of *Macrophomina phaseolina.*

The full growth of both fungi in the control samples from day 1 to 5 shows their pattern of growth on a normal Potato Dextrose Agar (PDA) without limonene. Therefore, the biological activities described above for both cultures treated with 1 ml of limonene and 9 ml of potato dextrose agar (PDA) using the poisoned food technique indicate positive inhibition effect of limonene against the two fungal species (*Trichoderma harzianum* and *Macrophomina phaseolina*) used in this study and the diameter of growth in inhibition zones ranged from 1.50 mm to 2.10 mm. Also percentage inhibition of limonene against both fungal species using same amount of limonene (1 ml) was 95% against *T. harzianum* and 89% against *M. phaseolina.*

Essential oils from different plant parts are known for their antimicrobial activities but the antifungal effects of essential oil from citrus species may vary with different parameters such as specific organisms used, extraction conditions, antifungal test method, chemicals used, geographical distribution, etc. This could also affect different measured inhibition values. Also fungus families express different behaviours towards the same tested essential oils (Souza et al. 2005, Jing et al. 2014). The results obtained in this study are in agreement with the inhibitory effects reported by Singh et al. (2010) where limonene and citral combination (1:1) strongly inhibited *Helminthosporizium oryzae* and *Trichoderma viridae* (100% inhibition) and Tao et al. (2014) where limonene inhibited *Penicillium italicum* (100% inhibition) as shown in Table 5.

Other similar studies are as summarised in Table 5. The table shows that the quantitative and qualitative differences of essential oil fractions and their antifungal capacities varied according to the distribution of the components of each fraction.

Table 5: Previous studies on antimicrobial investigation of citrus essential oils and extracts

Location	Citrus species studied	Plant part used for extraction	Substance tested	Fungi species tested	Method used	Results obtained	Reference
New York	*Citrus sinensis* Osbeck	Orange peels	Orange oil	*Salmonella senftenberg*	Filter paper disc method	93% inhibition	Dabbah et al. (1970)
Brazil	*Citrus limon* Burm.		Citral, eugenol	*Aspergillus niger*	Agar diffusion method	Inhibition halo = 10 mm	Souza et al. (2005)
India	*Citrus sinensis* Osbeck	Orange peels	Limonene (84.2%)	*Aspergillus niger*	Poisoned food assay Volatile activity assay	100% inhibition at 2.5 to 3.0 µg/ml of oil. These concentrations were fungicidal under the test conditions. *MIC = 3.0 µg/ml	Sharma and Tripathi (2008)
India	*Citrus reticulata* Blanco	Mandarin peels	Limonene (46.7%), geranial (19.0%)	*Alternaria alternata* *Rhizoctonia solani* *Curvularia lunata* *Fusarium oxysporum* *Helminthosporium oryzae* (*Cochliobolus miyabeanus*)	Poisoned food assay, volatile activity	*MIC = 0.2 ml/100 ml (0.2%) MIC > 0.2 ml/100 ml	Chutia et al. (2009)
India	*Citrus sinensis* Osbeck	Orange peels	Limonene (31.83%), citral (31.0%)	*Helminthosporium oryzae*	Poisoned food assay	Broad fungitoxic spectrum. 100% inhibition at 750 ppm. *D*-Limonene inhibited aflatoxin B_1 at 250 ppm. IC_{50} of *C. maxima* and *C. sinensis* oils were 8.84 and 9.45 µl/ml, respectively.	Singh et al. (2010)
	Citrus maxima Burm.	Pummelo peels	Limonene (31.83%), citral (31.0%)	*Helminthosporium oryzae*	Poisoned food assay		
	Citrus sinensis Osbeck	Orange peels	Limonene (31.83%), citral (31.0%)	*Trichoderma viride*	Poisoned food assay		
	Citrus maxima Burm.	Pummelo peels	Limonene (31.83%), citral (31.0%)	*Trichoderma viride*	Poisoned food assay		

Table 5 (Ctd)

Location	Citrus species studied	Plant part used for extraction	Substance tested	Fungi species tested	Method used	Results obtained	Reference
China	*Citrus sinensis* Osbeck	Orange peels	Limonene (77.49%), myrcene (6.27%)	*Penicillium chrysogenum*	Agar diffusion method, broth microdilution	Inhibition zone = 18.99 mm, *MIC = 9.33 µL/mL	Tao et al. (2009)
China	*Citrus reticulata* Blanco	Manadrin peels	Limonene (60.47%), γ-terpinene (0.04%)	*Penicillium italicum* *Penicillium digitatum*	Poisoned food assay	100% inhibition at 2.5 µL/mL	Tao et al. (2014)
Vietnam	*Citrus sinensis* Osbeck	Orange peels	Limonene (90.42%), myrcene (2.81%)	*Mucor hiemalis*	Agar dilution method	36.5% inhibition at 2000 ppm	Van Hung et al. (2013)
	Citrus aurantifolia	Lemon peels	Limonene (41.4%), β-pinene (18.54%)	*Mucor hiemalis*	Agar dilution method	100% inhibition at 2000 ppm	
	Citrus grandis Osbeck	Pummelo peels	Limonene (70.46%), γ-terpene (11.09%)	*Mucor hiemalis*	Agar dilution method	42.1% Inhibition at 2000 ppm	
Nigeria	*Citrus sinensis*	Orange peels	Aqueous extracts	*Aspergilus niger*, *Alternaria alternata*	Agar diffusion method	Mean zones of inhibition of *Aspergillus niger* and *Alternaria alternata* with the orange peel extracts were 0.33 ± 0.33 cm and 0.50 ± 0.50 cm, respectively	Oladele et al. (2019)

*MIC = Minimum inhibitory concentration

Conclusion

The extraction of limonene from orange peels by steam distillation method gave oily liquid which was distilled to give 95% limonene. The FT-IR result also confirmed the functional group present in the limonene. Also, GC-MS analysis confirmed molar mass of the extracted limonene to be 138 g/mol. Anti-fungi analysis showed that the limonene has strong inhibitory properties on *Trichoderma harzianum* and *Macrophomina phaseolina.*

Recommendation

The inhibition effect on limonene oil using the fungi, *Trichoderma harzianum* and *Macrophomina phaseolina* have been reported in this study. However, preparation of series of derivatives of limonene to established structure-activity relationship needs further study.

References

Amanzadeh Y, Ashrafi M and Mohammadi F 2006 New elaborated technique for isolation and purification of limonene from orange oil. *Iranian J. Pharm. Sci.* 2(2): 87-90.

Barceloux DG 2008 Citrus oil and limonene. In: *Medicinal Toxicology of Natural Substances*, pp. 635, John Wiley and sons Inc.

Chutia M, Bhuyan PD, Pathak MG, Sarma TC and Boruah P 2009 Antifungal activity and chemical composition of *Citrus reticulata* Blanco essential oil against phytopathogens from North East India. *LWT-Food Sci. Technol.* 42(3): 777-780.

Falk-Filipsson A, Bard J, Karlsson S and World Health Organization 1998 Concise International Chemical Assessment Document (CICAD) 5 Limonene, World Health Organization, Geneva, pp 8-18.

Dabbah R, Edwards VM and Moats WA 1970 Antimicrobial action of some citrus fruit oils on selected food-borne bacteria. *Appl. Environ. Microbiol.* 19(1): 27-31.

Đurović-Pejčev R, Potočnik I, Milijašević-Marčić S, Todorović B, Rekanović E and Stepanović M 2014 Antifungal activity of six plant essential oils from Serbia against *Trichoderma aggressivum f. europaeum*. *Pest. Phytomed.* 29(4): 291-297.

Ezejiofor TIN, Eke NV, Okechukwu RI, Nwaoguikpe R and Duru ME 2011 Waste to wealth: industrial raw materials potential of peels of Nigerian sweet orange (*Citrus sinensis*). *African J. Biotechnol.* 10(33): 6257-6264.

Ikhuoria EU and Gbenga GE 2007 Epoxidation and hydroxylation of African oil bean (*Pentaclethrea Marophylla*) seed oil. *J. Chem. Soc. Nigeria* 32(2): 264-268.

Javaid A and Rehman HA 2011 Antifungal activity of leaf extracts of some medicinal trees against *Macrophomina phaseolina*. *J. Medicin. Plants Res.* 5(13): 2868-2872.

Jing L, Lei Z, Li L, Xie R, Xi W, Guan Y, Sumner LW and Zhou Z 2014 Antifungal activity of citrus essential oils. *J. Agric. Food Chem.* 62(14): 3011-3033.

Katsuki T and Sharpless KB 1980 The first practical method for asymmetric epoxidation. *J. Am. Soc*. 102 (18): 5974-5976.

Koshle S, Mahesh S and Swamy SN 2016 Isolation and identification of *Trichoderma harzianum* from groundwater: An effective biosorbent for defluoridation of groundwater. *J. Environ. Biol.* 37(1): 135-140.

Ludwiczuk A, Skalicka-Woźniak K and Georgiev MI 2017 Terpenoids In: *Pharmacognosy: Fundamentals, Applications and Strategies*, pp 233-266, Academic Press.

Mohana DC and Raveesha KA 2007 Anti-fungal evaluation of some plant extracts against some plant pathogenic field and storage fungi. *J. Agric. Technol.* 4(1): 119-137.

Obidi OF, Adelowotan AO, Ayoola GA, Johnson OO, Hassan MO and Nwachukwu SCU 2013 Antimicrobial activity of orange oil on selected pathogens. *Int. J. Biotechnol.* 2(6): 113-122.

Oladele OO, Blessing DJ and Okosodo JI 2019 Antifungal activity and phytochemical analysis of selected fruit peels. *J. Biol. Med.* 3(1): 040-043.

Pawar VC and Thaker VS 2006 In vitro efficacy of 75 essential oils against *Aspergillus niger*. *Mycoses* 49: 316-323.

Plumlee KH 2013 Citrus Oil: *Small Animal Toxicology*, Elsevier Inc, pp 513-515.

Sharma N and Tripathi A 2008 Effects of *Citrus sinensis* (L.) Osbeck epicarp essential oil on growth and morphogenesis of *Aspergillus niger* (L.) Van Tieghem. *Microbiol. Res.* 163(3): 337-344.

Silverstein RB, Bassler GC and Morrill TC 1974 Spectrometric identification of organic compounds. 5th ed, John Wiley and sons, New York.

Singh P, Shukla R, Prakash B, Kumar A, Singh S, Mishra PK and Dubey NK 2010 Chemical profile, antifungal, antiaflatoxigenic and antioxidant activity of Citrus maxima Burm. and *Citrus sinensis* (L.) Osbeck essential oils and their cyclic monoterpene, DL-limonene. *Food and Chem. Toxicol.* 48(6): 1734-1740.

Souza ELD, Lima EDO, Freire KRDL and Sousa CPD 2005 Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. *Brazilian Arch. Biol. Technol.* 48: 245-250.

Tao NG, Liu YJ and Zhang ML 2009 Chemical composition and antimicrobial activities of essential oil from the peel of bingtang sweet orange (Citrus sinensis Osbeck). *Int. J. Food Sci. Technol.* 44(7): 1281-1285.

Tao NG, Jia L and Zhou HE 2014 Anti-fungal activity of *Citrus reticulata* Blanco essential oil against *Penicillium italicum* and *Penicillium digitatum*. *Food Chem.* 153: 265-271.

Van Hung P, Chi PTL and Phi NTL 2013 Comparison of antifungal activities of Vietnamese citrus essential oils. *Nat. Prod. Res.* 27(4-5): 506-508.