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ANTIMICROBIAL ACTIVITIES OF ESSENTIAL OILS FROM *Peperomia pellucida* (Linn.) LEAF OBTAINED IN NIGERIA

A. O. Ogunmoye 1[1], I. A. Oladosu 2[3], O. C. Atewolara-Odule 3[1*], O. O. Olubomehin 4[1], I. B. Onajobi 5[2], S. Tijani 6[1]

[1]Department of Chemical Sciences, Olabisi Onabanjo University, P. M. B. 2002, Ago-Iwoye, Ogun-State, Nigeria.
[2]Department of Microbiology, Olabisi Onabanjo University, P. M. B. 2002, Ago-Iwoye, Ogun-State, Nigeria.
[3]Chemistry Department, University of Ibadan, Nigeria.

*Corresponding Author. E-mail: atewolara-odule.odunayo@oouagoiwoye.edu.ng
Phone no.: 08054622445 or 08054646906

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ABSTRACT

Peperomia pellucida (Linn.) is a shiny or silver coloured bush belonging to Piperaceae family. Ethno-medicinal uses of this plant has been applied for treating abdominal pain, abscesses, acne, boils, colic and fatigue. The essential oil was extracted from the air-dried leaves of *P. pellucida* by hydrodistillation method using a Clevenger apparatus. The essential oil obtained was light yellow with offensive odour and a yield of 0.30 v/w. Atotal of thirty (30) components were identified and the major constituents are Elemol (9.32%), Neointermedeol (8.35%), 1H-3a,7-Methanoazulene- (5.60%) and Bicyclo [2.2.1] heptanes,2,2,3-trimethyl (5.08%). Sesquiterpenes and oxygenated sesquiterpenes (52.71%) made up a significant portion of the leaf oil. The antimicrobial effects showed it has Minimum Inhibition Concentration (MIC) on *Pseudomonas aeruginosa* and *Bacillus subtilis* at 0.01% oil concentration having 7.0 mm and 9.3 mm zones of inhibitions; but MIC against *Bacillus cereus* was obtained at 0.1% concentration of the oil. The result of antifungal on *Lasiodiplodia theobromae*, *Fusarium oxysporum* and *Aspergillus tamari* at different concentration of 1.0, 0.1, 0.01, 0.001 and 0.0001% showed that the essential oils from the plant has potent antifungal effects on the three fungi species. These results showed that essential oil from the plant could act as potential antimicrobial agent.

Keywords: *Peperomia pellucida*, Hydrodistillation, GC-Mass Spectroscopy

INTRODUCTION

Peperomia pellucida (Linn.) is a shiny bush or silver bush belonging to Piperaceae family.Ethno-medicinal uses of this plant has been applied for treating abdominal pain, abscesses, acne, boils, colic, fatigue, gout, headache, renal disorders, and rheumatic joint pain[1].Phytochemical screening of the plant revealed the presence of alkaloids, cardenolides, saponins and tannins, while anthraquinones was absent [2].The essential oil of *P. pellucida*showed antibacterial, anti-inflammatory and analgesic activities [3].The aim of this study is to investigatethe chemical properties and antimicrobial activities of the essential oil of *Peperomia pellucida*.

MATERIALS AND METHODS

Plant materials

*P. pellucida*leaf was collected from the farm land in Olabisi Onabanjo University, Ago- Iwoye. It was identified at the Department of Plant Science and Applied Zoology, Olabisi Onabanjo University.

Extraction of the Essential Oils

The air-dried(180 g) of the leaf was hydro-distilled in an all glass Clevenger-type apparatus for 4 h in accordance with British Pharmacopoeia method [4].

Gas chromatography (GC) and Gas chromatography-mass spectrometry (GC-MS)

The essential oils were subjected to GC analysis on a Hewlett Packard HP model 6820. A gas chromatograph fitted with a flame ionization detector (FID) and DB-5 (60m x 0.25 mm id, 0.25 µm film thicknesses and the split ratio was 1:2). Hydrogen was used as carrier gas at 99.99% purity on a stationary phase column (HP5 MS). The GC oven temperature was programmed at 50°C (held for 2 min) at 5°C/min heated to 240°C (held for 10 min). Injector and detector temperatures were fixed at 200°C and 240°C respectively. An aliquot (0.5 µL of the diluted oil) was injected into the GC.

The GC-MS analyses were performed on a Hewlett Packard model 5973 mass spectrometry system equipped with a DB-5 capillary column (30 m x 0.25 mm id, film thickness 0.25 µm) under the same condition as the GC column. The oven temperature was programmed from 70-240 ^{o}C and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 mL/min. the scanning range was 35 to 425 amu. Diluted oil in n-hexane was injected into the GC/MS.

Identification of components

Identification of the essential oil components was based on their retention indices and by comparison of their mass spectral fragmentation patterns in computer matching against in-built data (NIST database, Wiley library mass spectra database, pherobase).

Test Microorganism

The tests microorganisms (*Lasiodiplodia theobromae*, *Fusarium oxysporum and Aspergillus tamari*) and the bacteria specimens (*Pseudomonas aeruginosa*, *Bacillus cereus* and *Bacillus subtilis*) used were collected from Plant Science laboratory (Pathology research laboratory) Olabisi Onabanjo University, Ago-Iwoye. The prepared pure cultures of the tests microorganisms were preserved at the room temperature.

Preparation of Media

Potato Dextrose Agar (PDA) was used for the fungal (pathogens) assay. PDA was prepared by weighing 19.5 g of potato dextrose agar and was dissolved in 500 mL of distilled water in a 500 mL capacity conical flask and covered with foil paper which was then sterilized by autoclaving at 121°C for 15 minutes.

Preparation of Antifungal Assay

The antifungal assay was carried out in the Petri dishes (90 mm in diameter) containing PDA. When temperature of the medium (PDA) reached about 43^{o}C, specific initial concentration of plant essential oils (diluted in ethanol 96%) were made into five (5) concentrations; 1.0%, 0.1%, 0.01%, 0.001% and 0.0001% and each of the concentration was added into already prepared PDA and mixed thoroughly in separate Petri-dishes with their respective concentrations. For control set, sterilized water was used in place of the oil and was added to the medium. The PDA containing essential oils on 9 cm Petri dishes were then inoculated with mycelium taken from the periphery of 5-days old stock cultures of the test fungi species (specimen) and incubated. The plugs of mycelium were removed with a 6 mm diameter cork borer, inverted and where placed in the center of each Petri dish. Plates were sealed with paper tape (masking tape). Three replicate plates were sited up for each concentration, and the

plates incubated in the dark at 26 ± 2°C. The rate of mycelia growth inhibition was measured after placing an active seven days mycelia plug of fungi on Petri dishes containing PDA. The observations were recorded after 2-4 days. The rate of mycelia growth inhibition (GI %) was calculated using the following formula: *G.I % = dc – dt/dc x 100*

Where dc is mean colony diameter of control sets and dt is mean colony diameter of treatment sets. The experiments were conducted in a completely randomized design (CRD) with five concentrations and three replicates.

Agar Diffusion: Pour Plate Method for Bacteria

Nutrient agar and Nutrient broth were used for bacteria assay. For Nutrient agar, 7.0 gram of nutrient agar was dissolved in 250 mL of distilled water in a conical flask and covered with foil paper which was then sterilized by autoclaving at 121°C for 15 minutes. After autoclaving, it was allowed to cool to about 45°C and subsequently dispensed into petri-dishes under sprit lamp to avoid contamination. The plates were allowed to gel and about 15 mL from already cooled nutrient agar medium was dispensed in each of the petri-dishes and allowed to gel. Nutrient broth- 5.0 mg of nutrient broth medium was weighed into 200 mL conical flask and autoclaved at 121°C for 15 minutes. This medium was allowed to cool to about 43°C and dispensed into sterile McCartney bottles. Pure culture of the three bacterial species were inoculated into the already prepared bottle containing nutrient broth, labeled and left to grow overnight. The different concentrations of the essential oil at 1.0%, 0.1%, 0.01% and 0.001% were inoculated into their respective well on different plates of the bacteria species at the number site under the plates. The plate's haven inoculated them with the plant oil concentrations were incubated for another 24 h. Observation was made after 24h and the Inhibitory zones recorded for those concentrations.

RESULTS AND DISCUSSION

The yield of volatile oil from *P. pellucida* was0.30% v/w. From the GC-MS analysis, 30 constituents were identified (Table 1) among which elemol (9.32%), Neointermedeol (8.35%), 1H-3a,7-Methanoazulene- (5.60%) and Bicyclo [2.2.1] heptanes,2,2,3-trimethyl (5.08%) were the major components. The oil was dominated by sesquiterpenes and oxygenated sesquiterpenes (52.71%). The main constituents previously reported in literature from Brazil were dillapiole (39.7%) and (*E*)-caryophylene (10.7%) [5], Gamma-gurjunene (11.34%±0.02), 1,10-di-epicubenol (11.27%±0.02), (*E*)-caryophylene (8.71%±0.02) and dillapiole (8.50%±0.03) [6].The variation may be attributed to difference in geographical location. [7]

Antimicrobial activity

In vitro antimicrobial effects of the essential oil of *P. pellucida* showed a range of growth inhibition pattern against pathogenic microorganisms with the increase in the concentration of the oil (Table 2). *Bacillus subtilis* was noticed in this study to be highly susceptible to the antibacterial potential of the plant oil having 11.6 mm and 17.3 mm inhibitory zones respectively. This shows that the oil has the potential to control the pathogens.

Table 1: The identified Compound Names and Retention Index Comparisons

S/N	COMPOUND NAME	$RI_{cal.}$	% Composition
1	Terpinen-4-ol	729±0	1.31
2	Durenol	1000±0	2.23
3	p-Thymol	1012±0	0.08
4	α.- copaene	1422±0	2.11
5	β- copaene	1500±0	3.00
6	Humulene	1500±0	4.10
7	Beta Eudesmene	1500±0	4.04
8	Caryophyllene oxide	1500±0	0.68
9	Norbornane	1500±0	5.08
10	Caryophylladienol II	1243±0	4.15
11	Neointermedeol	1500±0	8.35
12	Patchoulane	1463±0	5.60
13	3-(2- Isopropyl-5-methylphenyl) -2-m ethyl propionic acid	1500±0	3.47
14	Beta Costol	1484±0	9.32
15	Aromadendrene oxide-(1)	1500±0	1.02
16	Alpha-Cyperone	1519±0	1.66
17	Hexahydrofarnesyl acetone	1155±0	2.52
18	Ethanone,1-6,6-dimethylbicyclo[3.1.0]	1177±0	1.42
19	P-fluoro -alpha- methyl styrene	1000±0	1.33
20	3,4-Xylenol,6-ethyl	1542±0	3.52
21	Butylphosphonic acid,4-isopropylphenyl propyl ester	1000±0	2.90
22	2H-Inden-2-one,1,4,5,6,7,7a,-hexahydro-7a-methyl-,(S)-	1204±0	2.06
23	2,4-Mesitylene diamine	1819±0	3.63
24	Phytol	1197±0	3.13
25	*2*-Methyl-5-(3,4-methylenedioxycinnamoyl)pyridine	1926±0	3.12
26	Benzene,1,1'-(1,2-ethynediyl)bis 2,4-dimethoxy	1744±0	4.46
27	Phytol,acetate	1357±0	1.55
28	4'-propoxy-2-methylpropiophenon	2217±0	2.13
29	Chol-7-ene,(5.beta.)-	1426±0	3.47
30	4,11-Selinadiene	2477±0	1.73

Key: $RI_{cal.}$ = Retention Index calculated.

Table 2: The Antibacterial Effects of *Peperomia pellucida of* Essential Oil on The Bacterial Pathogens

Bacterial Species	Average Diameter Zones of Inhibition (mm) on the Bacteria Species				
	1.0 %	0.1 %	0.01 %	0.001 %	**Streptomycin**
Pseudomonas aeruginosa	11.0	9.3	7.0	-	**32.0**
Bacillus cereus	10.6	8.0	-	-	**29.3**
Bacillus subtilis	17.3	11.6	9.3	-	**18.7**

The antifungal activities also showed a range of growth inhibition pattern against pathogenic microorganisms with the increase in the concentration of the oil (Table 3).The highest percentage inhibitions was observed on *Lasiodiplodia theobromae* at 24 hours of incubation. These results on the pathogens showed that the plant oil could serve as antifungal agent but at concentration not below 0.1%.The essential oil showed appreciable high percentage inhibition at 1.0% and 0.1% concentration. The essential oil was noticed to have better percentage inhibition at 1.0% concentration of the essential oil against all the three fungi used. This could also be due to the chemical composition of the essential oil which has been reported to have antibacterial action against some microbes. The antimicrobial activity of the leaves of *Peperomia pellucida* had previously been reported [8].

Table 3: Percentage Growth Inhibition (GI) of the Three Fungal Species

Fungi	Average Diameter Mycelia Growth (mm) of the Fungal Species at Different Hours of Treatment in Percentages (%)						
	Hours of Interval	**Control**	**1.0%**	**0.1%**	**0.01%**	**0.001 %**	**0. 0001%**
Aspergillus tamarii	**24**	38.0	84.2	84.2	73.6	65.7	52.6
	48	45.0	72.9	73.3	60.4	52.1	41.6
	72	60.0	83.3	80.0	36.7	28.3	13.3
	96	90.0	86.7	64.4	38.9	37.7	33.3
	120	90.0	76.7	55.5	27.8	18.9	5.55
Lasiodiplodia theobromae	**24**	25.0	100.0	100.0	80.0	72.0	68.0
	48	36.0	83.0	72.0	66.7	66.7	55.5
	72	66.0	87.8	72.7	54.5	54.5	31.8
	96	90	88.8	75.5	55.5	55.5	33.3
	120	90.0	88.8	60.0	44.4	44.4	16.7
Fusa	**24**	6.0	33.3	33.3	33.3	33.3	33.3

48	10.0	60.0	40.0	50.0	40.0	0.0
72	12.0	68.7	68.7	18.7	12.5	0.0
96	26.0	80.7	61.5	53.8	50.0	23.0
120	30.0	70.0	60.0	50.0	40.0	26.6

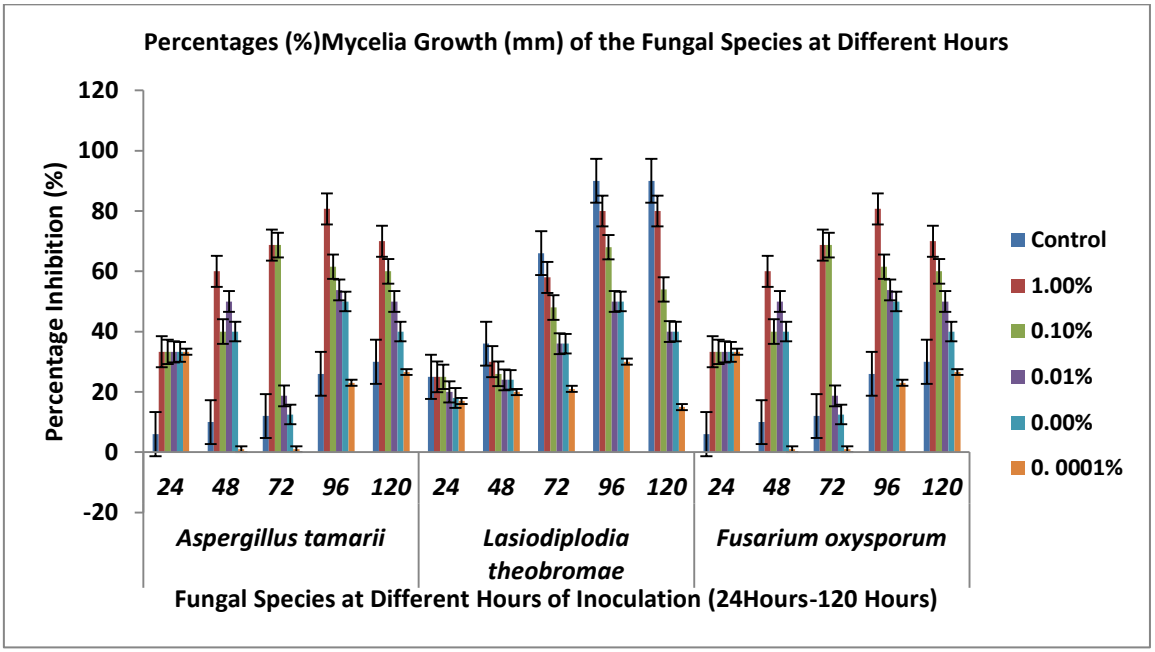

Figure 4: Percentages (%) Mycelia Growth (mm) of the Fungal Species at Different Hours

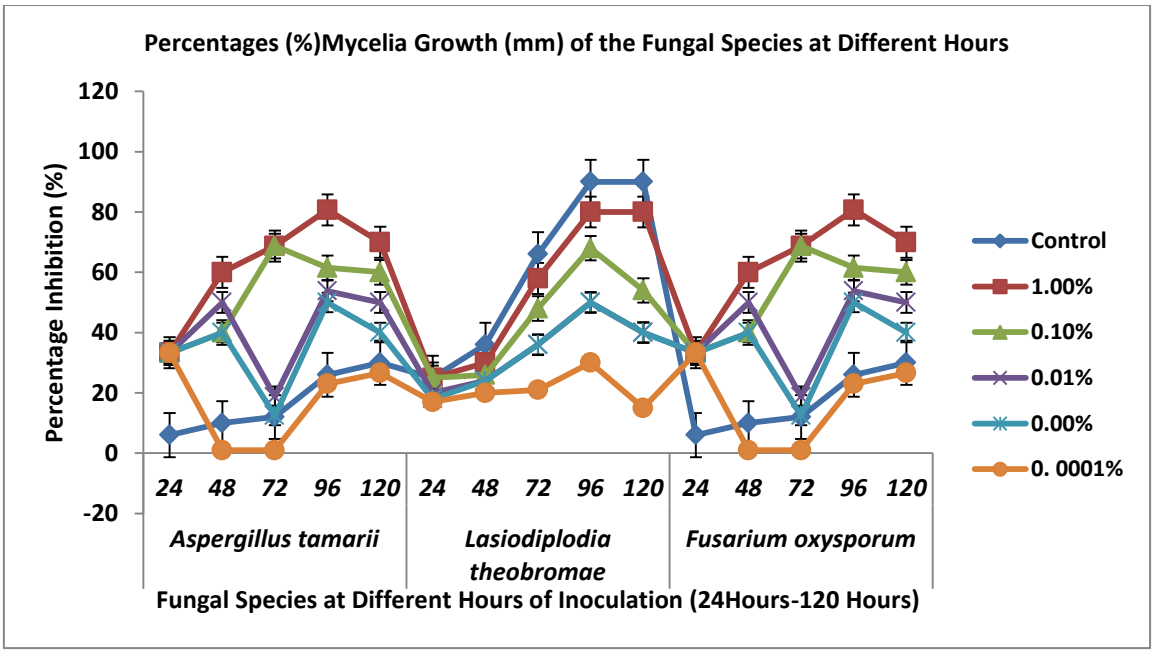

Figure 5: Percentages (%) Mycelia Growth (mm) of the Fungal Species at Different Hours showing the comparison of the anti-fungi effect of the essential oil on the three fungi species.

CONCLUSION

The results showed that this plant oil could act as a potential antiseptic agent; however, further investigation should be carried out against new series of pathogenic microorganisms. This oil can be classified as medicinal because of its high sesquiterpenes content. This probably provides some pharmaceutical rationale for the popular use of this plant in traditional herbal medicine.

REFERENCES

1. G.K. Oloyede, P.A. Onocha and B.B Olaniran (2011). Phytochemical, toxicity, antimicrobial and antioxidant screening of leaf extracts of *Peperomia pellucid* from Nigeria. *Adv. Environ. Biol.*, 5(12): 3700-370

2. M.P. Mishra (2010). *Peperomia pellucida*, an Amazing Wild Medicinal Herb Ecosensorium Org. http://www.ecosensorium.org/2010/11/peperomia-pellucidaamazing-wild.html.

3. Khan, M.R., Omoloso, E. D.(2002). Antibacterial activity of *Hygrophila stricta a*nd *Peperomia pellucida Fitoterapia* 3, 251–254.

4. British Pharmacopoeia, (1980) H.M. Stationary Office Vol II.

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5. M.H.L. Da Silva, M.D.G.B. Zoghbi, E.H.A. Andrade and J.G.S. Maia. (1999). The essential oil of *Peperomia pellucida* Kunth and P. circinnata Link var. circinnata. *Flavour Fragr. Journal*., 14: 312-314.

6. J.C.S. De Oliveira, C.A.G. Da Camara, R.C.S. Neves andP.S. Botelho (2017). Chemical composition and acaricidal activity of essential oilsfrom *Peperomia pellucida* Kunth. against *Tetranychus urticae*. *Rev. Virtual Quim*. *9* (6), 2204-2213.

7. R. Cooper and G. Nicola (2015) Natural Products Chemistry: Sources, Separations and Structures. CRC Press, Taylor & Francis Group, New York, 15-17.

8. Khan A, Rahman M and Islam S. (2010). Isolation and bioactivity of a xanthone Glycoside from *Peperomia pellucida*. *Life Sciences and Medical Research*, 1**,** 1-10.