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GC-MS Evaluation of Palm Oil as Benign Extraction Medium for Bioactive Constituents of *Ocimum gratissimum* L and *Bryophyllum pinnatum* (Lam.)

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Abstract: Medicinal and aromatic plants have huge potential for the discovery of novel bioactive molecules. Th e use of medicinal plants especially in primary health care has become popular leading to increasing search for p lants with therapeutic potential. This work is aimed at evaluating palm oil as green extraction medium for bioact ive constituents of *O. gratissimum* and *B. pinnatum* leaves. The essential oil (EO) of the leaves of *Ocimum grati ssimum* (OG) and *Bryophyllum pinnatum* (BP) were obtained through hydrodistillation and palm oil extraction. The extracts were analysed by gas chromatography mass spectrometry (GC-MS). Thymol (19.27%), *o*-cymene (9.05%), caryophyllene (6.55%), caryophyllene oxide (6.54%), terpinen-4-ol (6.14%) and β-eudesmene (6.01%) were the major compounds in O. *gratissimum.* The result of GC-MS analysis of the essential oil of *B. pinnatum* showed fifty seven different compounds with nonanal (17.3%) and geranylacetone (13.1%), and spathulenol (3. 6%) as the major compounds. The palm oil extract of the leaf of *O. gratissimum* on GC-MS analysis showed tha t it selectively extracted thymol, whereas the palm oil extract of leaf of *B. pinnatum*s selectively extracted spathu lenol, and hexadecylinedane. These compounds may be responsible for the use palm oil extract of leaf of *B. pinn atum* for the treatment of severe ear ache.

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Keywords: Essential oil, *Ocimum gratissimum*, *Bryophyllum pinnatum*, palm oil, bioactivity

1. Introduction

Medicinal and aromatic plants have huge potential for discovery of novel bioactive molecules (Mbakwem-Aniebo *et al*., 2012). The use of medicinal plants especially in primary health care has become popular leading to increasing search for plants with therapeutic potential (John *et al*., 2011). Several infectious diseases have been managed with herbal remedies (Buba *et al*., 2016). Medicinal and aromatic plants are used for treatment of various infections in Nigeria (Mbakwem-Aniebo *et al*., 2012). Research revealed that some medicinal plant constituents are effective against a wide range of disease causing pathogens (Mbakwem-Aniebo *et al*., 2012). Approximately 25% of modern dugs and 60% of antitumor drugs were derived from naturally occurring products. Medicinal plants are used in different countries including India, China, and Nigeria. To ascertain and guaranteed the quality and effectiveness of herbal plants, certain pharmacopoeias steps are adopted which includes, correct identification of the plant species among others (Prablu *et al*., 2009).

Ocimum gratissimum is a herbaceous plant which belongs to the family of Labiatae/ Lamiaceae. It is commonly known as scent leaf or clove basil and is distributed in many countries including Nigeria, where the palm oil extract is used for healing of wounds, joint pain and edema (Prablu *et al*., 2009). The plant has been identified with different names in different part of the world, some of the most commonly used one are viriddhuhtisul by Sanskrit, Ramtsulsi by Hindi, Nimmatulasi by Kannada. In Nigeria *Ocimum gratissimum* has also been differently identified by different tribes with different vernacular names. In the middle belt part of Nigeria the plant is called ‘’ojujuokpebwu’’ by igede speaking tribe,’’efinrinnla’’ by Yoruba speaking

tribe,'' Aramogho'' by Edo speaking tribe while in Hausa and Igbo speaking tribe it is called Daidoya and Ahuji respectively (Prablu *et al*., 2009). Other species of the plant are *Ocimum viride Linn, Ocimum suave Linn, Ocimum basilicum Linn*, and *Ocimum canum sim* (Prablu *et al*., 2009).

The plant *Bryophyllum pinnatum* (crussalaceac) on the other hand is commonly known as air plant, love plant, miracle leaf, and life plant (Jain, 2010). The plant has been accepted as a herbal remedy in many parts of the world (Igwe and Akanyuti, 2005). It is a crussalescents herb of about 1metre in height, with opposite glabrous leave (with 3-5 deeply crenulated, fleshly leaflets) (Ojiwole, 2002). It is distributed worldwide (Yanx and Yamagishi, 1992). It grows widely and used as folk medicine in America, Madagascar, Asia, Hawaii and Africa (Yadav and Dixit, 2003). It is astringent, sour in taste, sweet in the post digestive effect and has a hot potency. It is well known for its haemostatic and wound healing properties. The plant has considerable attention for its medicinal properties and application in folk medicine, as well as in contemporary medicine (Kamboj and Salaja, 2016). The leaves and bark of *B. pinnatum* are bitter tonic, astringent, analgesic and carminative, ethno pharmacologically used for treatment of diarrhea, voting, earache, burns, abscesses, gastric ulcers, insect bites and lithiasis. Juice from the leaves is used for the small pox, ear ache, otitis, cough, asthma, palpitations, headache, convulsion, and general debility (Jain, 2010). The plant has also been employed for the treatment of edema of legs (Okwu and Nnamdi, 2011). Leaf powder is used for wound dressing in south-eastern Nigeria. The herb is used to facilitate dropping of the placenta of newly born baby (Dalziel and Hutchinson, 1995). It is also applied to the body of young children when they are ill. It is largely used in folk medicine for the treatment of hypertension and kidney stone (Lans, 2006), pulmonary infections and rheumatoid arthritis. In traditional medicine, the leaf of the plant has been used as antifungal (Majaz *et al*., 2011), potent antihistamine and anti-allergic activity.
This study aims at the evaluation of palm oil as benign extraction medium for bioactive constituents of *Ocimum gratissum* and *Bryophyllum pinnatum* in comparison to Hydrodistillation.

2. Materials and Methods.

Chemicals and solvents

Dichloromethane GC grade was purchased from Sigma-Aldrich (Germany). Palm oil was purchased from commercial store in Abuja.

Collection and processing of plant sample.

The leaves of *Ocimum gratissimum* and *Bryophyllum pinnatum* were collected from NIPRD medicinal plant garden, at Idu industial Area, Abuja FCT, Nigeria, in October 2018. The leaves of *O. gratissimum* were air-dried for seven days. The dried leaves of *O. gratissimum* were coarsely pounded and soaked (macerated) with palm oil for 24hours at 40^{o}C, filtered to obtain the *Ocimum gratissimum* palm oil extract (OGPOE).
Fresh leaf of *Bryophyllum pinnatum* was cleaned to remove dirt, slightly rubbed with palm oil (PO) and mildly heated at 60^{o}C to soften the leaf. The soften leaf was squeezed and the juice extracted to obtain *Bryophyllum pinnatum* palm oil extract (BPPOE)

Isolation of Essential Oils by Hydrodistillation

Fresh leaves of *Ocimum gratissimum* and *Bryophyllum pinnatum* were chopped into pieces and the materials were separately hydrodistilled for 4 hours using Clevenger-type apparatus, to obtain the essential oils.

Gas Chromatography–Mass Spectrometry (GC-MS) analyses

The essential oils and palm oil extracts were 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 45-400 amu, and scan rate of 3.99 scans/sec], and Shimadzu LCsolution data system. The Gas chromatography column was HP-5MS fused silica capillary with 5% phenyl-methylpolysiloxane 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 60-180°C at a rate of 10°C/min, then held at 180°C for 2 min, followed by 18-280°C at a rate of 15°C/min, then again held at 280°C for 4 min. The injection port temperature was 250°C, while detector temperature was 280°C. Diluted sample (1/100 in hexane, v/v) and 1.0 µL was injected using autosampler and in the split mode with ratio of 10:90. Individual constituents were identified by comparing their mass spectra with known compounds and NIST Mass Spectral Library (NIST). The percentages of each component are reported as raw percentages based on the total ion current. The results of GC-MS analyses are shown in Tables 1 and 2.

3. **Results and Discussion**.

Table 1: Volatile constituents obtained from hydrodistillation and palm oil extract of *Ocimum gratissimum* leaf

S/N	**Compound Names**	**% Composition**		
		OGEO	OGPOE	PO
1.	α-Phellandrene	12.81	----	----
2.	Myrcene	1.32	----	----
3.	4-Carene	2.47	----	----
4	o-Cymene	9.05	----	----
5	γ-Terpinene	4.23	----	----
6	α-4-Dimethylstyrene	2.02	----	----
7	Bormeol	2.92	----	----
8	Terpinen-4-ol	6.14	----	----
9	Thymol methyl ether	1.11	----	----
10	Thymol	19.27	1.57	----
11	Carvacrol	1.95	----	----
12	Copaene	1.65	----	----
13	β-Elemene	0.79	----	----
14	Caryophyllene	6.55	----	----
15	α-Bergamotene	1.56	----	----
16	α-Humulene	2.52	----	----
17	β-Eudesmene	6.01	----	----
18	α-Selinene	3.99	----	----
19	Eudesma-3,7(11)-diene	2.50	----	----
20	Cryophyllene oxide	6.54	----	----
21	Alloaromadendrene oxide	0.72	----	----
22	α-cadinol	1.49	----	----
23	Spathulenol	1.29	----	----
24	Palmitic acid	1.09	39.88	46.42
25	Myristic	----	0.90	----
26	6-Otadecenoic acid	----	24.17	48.40
27	Trans-Oleic acid	----	16.26	----
28	Stearic acid	----	11.05	----
29	Arachidic acid	----	0.83	----
30	Fumaric acid,2-Dimethylaminoethy	----	0.62	----
31	2-Hexadecanoyl glycerol	----	2.18	----
32	E,E,Z-1,3,12-Nonadecatriene-5,14-d	----	2.53	----
33	n-Decanoic acid	----	----	5.18

Key: OGEO= *Ocimum gratissimum* essential oil; OGPOE=*Ocimum gratissimum* palm oil extract

Table 2: Volatile constituents obtained from hydrodistillation and palm oil extract of *Bryophyllum pinnatum* leaf

SN	**Compound Names**	**% Composition**			
		BPEO	**BPCPOE**	**BPE**	**PO**
1.	α-Phellandrene	0.30	----	----	----
2.	α-Pinene	1.31	----	----	----
3.	β-Terpinene	4.94	----	----	----
4.	β-Myrcene	2.89	----	----	----
5	β-Pinene	0.37	----	----	----
6.	Isoterpinolene	0.44	----	----	----
7.	o-Cymene	2.37	----	----	----
8.	Eucalyptol	11.32	----	----	----
9.	γ-Terpinene	4.18	----	----	----
10.	L-Fenchone	3.41	----	----	----
11.	Nonanal	0.80	----	----	----
12.	Fenchol	7.17	----	----	----
13.	Borneol	0.57	----	----	----
14.	L-4-Terpineol	0.75	----	----	----
15.	Terpenol	0.22	----	----	----
16.	Nonanoic acid	0.42	----	2.96	----
17.	Elixene	0.91	----	----	----
18.	8-Methyl-1-decene	0.22	----	----	----
19.	α-Cubebene	0.23	----	----	----
20.	β-Bourbonene	0.82	----	----	----
21.	β-Elemene	1.03	----	----	----
22.	Caryophellene	10.57	----	----	----
23	α-Bergamotene	3.68	----	----	----
24.	α-Caryophyllene	1.42	----	----	----
25	Curcumene	1.10	----	----	----
26	Germacrene D	2.11	----	----	----
27.	Alloaromadendrene	1.07	----	----	----
28.	Germacrene B	3.98	----	----	----
29.	1-Pentene,5-(2,2-dimethylcyclopropane	0.31	----	----	----
30.	8-Pentadecanol	0.35	----	----	----
31.	Spathulenol	3.60	0.88	----	----
32.	Caryophyllene oxide	4.35	----	----	----
33.	1-Undecyn-4-ol	0.31	----	----	----
34.	10,10-Dimethyl-2,6-dimethylenebicyclo[7.2.0]undecane	0.65	----	----	----

35.	Eudesm-7(11)-en-4-ol	0.87	----	----	----
36.	Ledo	0.35	----	----	----
37.	Patchoulane	6.06	----	----	----
38.	Bergamotol,Z-alpha-trans	0.63	----	----	----
39.	Alpha-Farnesene	1.54	----	----	----
40.	Cedr-8-en-13-ol	0.23	----	----	----
41.	n-Pentadecanol	0.61	----	----	----
42.	1,2-Benzenediol,o,o-dicylopropane	0.30	----	----	----
43.	n-Hexadecanoic acid	0.94	----	----	----
44.	Palmitic acid	0.32	26.05	28.01	37.88
45.	7,22-Erostadienol acetate	0.57	----	----	----
46.	Rimuene	2.38	----	----	----
47.	Deydroabietane	2.86	----	----	----
48.	1,3-Hexanedione, 1-phenyl-2,5-dimethane	0.22	----	----	----
49.	Cembrene	1.31	----	----	----
50.	1-Hexyl-1-nitrocyclohexane	0.32	----	----	----
51.	m-Toluic acid, undec-2-enyl ester	0.21	----	----	----
52.	2-Methyl-5-(furan-3-yl)-pent-1-en-3	0.17	----	----	----
53.	Citronellyl butyrate	0.15	----	----	----
54.	Diazoprogesterone	0.19	----	----	----
55.	Cholest-14-en-3-ol	1.19	----	-----	----
56.	Abieta-8,11,13-trien-18-ol	0.29	----	----	----
57.	1-Hexadecylindane	0.24	6.69	----	----
58	N-Acetylisoxazolidine	----	----	0.71	----
59	1-Hexadecanol	----	----	30.53	----
60	Capric acid	----	----	3.72	----
61	Neohexane	----	----	0.91	----
62	1-Iodononane	----	----	3.93	----
63	1-Iododecane	----	----	7.59	----
64	4,5-Octanediol	----	----	0.35	----
65	9-Eicosyne	----	----	21.29.	----
66	Methyl carbonate	----	0.77	----	----
67	Isobutyl-2-hydroxypropanoate	----	1.42	----	----
68	Propylene glycol ethyl ether	----	2.13	----	----
69	Oleylalcohol,trifluoroacetate	----	31.07	----	----
70	2-Hexadecanoyl glycerol	----	18.83	----	----
71	4-Pentenylcyclohexane	----	12.16		----

Key: BPEO = *Bryophyllum pinnatum* essential oil, BPPOE = *Bryophyllum pinnatum* palm oil extract, BPE = *Bryphyllum pinnatum* extract and PO = Palm oil.

Table 3: Chemotype in essential oil of *Ocimum gratissimum* grown in various countries

Origin	Chemotype	Major compounds
Brazil	Eugenol	Eugenol (54.8%), α-bisabolene (17.2%), thymol (9.8%) 1, 8-cineole (5.6-22.6%)
Colombia	eugenol	Eugenol (43.2%), 1,8-cineole (12.8%) and β-selinene (9.0%)
Nigeria	eugenol	Eugenol (61.9%), o-cymene (9.05%), cis-ocimene (8.2%), germacrene D (4.4%),
Rwanda	Thymol	Thymol (35%), ϒ-terpinene (23%) eugenol (11%)
Northern India	Eugenol	Eugenol (77.2%), 1, 8-cineole (7.6%), germacrene D (2.7%) and β-caryophyllene (1.7%)
Southern India	Phenylpropanoids	Eugenol (57.1%), phenylpropanoids (57.3%) α-bulnesene (15.6%) and β-caryophyllene (14.2%)
Algeria	Eugenol	Eugenol (54.8%), β-elemene (10.9%), 1, 8-cineole (4.1%)
Benin	P-cymene	P-cymene (28.1-53.8%), thymol (3.3-29.1%), ϒ-terpinene (1.1-10.9%), α-thujene (3.4-10.8%) and myrcene (4.2-8.3%)
India	Eugenol	Eugenol (84.8%), limonene (1.7%), methyl eugenol (1.5%), β-caryophyllene (1.2%), farnesene (1.1%), eugenol (75.1%), terpinolene (14.2%); eugenol (62.0-74.8%) and limonene + 1, 8-cineole (10.8-7.7%)
Kenya	Eugenol	Eugenol (68.8%), methyl eugenol (13.2%), cis-ocimene (7.5%), germacrene D (4.3%), and trans-crayophyllene (1.7%), eugenol (95.5-70.1%), Z-β-ocimene (34.15).
Portugal	Thymol	Thymol (48.1%) and p-cymene (12.5%)
Republic of guinea	Thymol	Thymol (46.0%), p-cymene (12.0%) and ϒ-terpen+t-sabiene hydrate (17.0%)
Romania	Linalool	Linalool (74.2%), eugenol (3.5%), and t-cadinol (3.5%)

4. Discussion

The chemical constituents of *Ocimum gratissimum* obtained from its leaves is known to contain essential oil with various pharmacological applications (Sandeep, 2017).Generally, the plant has antimicrobial, antioxidant, antidiarrheal, antimalarial and anticancer properties. Extensive research has revealed numerous important uses of *Ocimum gratissimum* in many countries. Generally *Ocimum gratissimum* can be used for treatment of mental illness, stomach ache, diarrhea, chronic dysentery and vomiting and other health benefits such as high fever, epilepsy, ear ache, colon cancer, antifungal, cold and cataarh (Ntezurubanza *et al*., 1987). Recent development has shown that *Ocimum gratissimum* is useful in the treatment of viral disease like human immunodeficiency virus (HIV), acquire immunodeficiency syndrome and vaginal douches and vaginitis (Siddhartha and Nag-handhuri, 1991). The chemical constituents of the essential oil of *Ocimum gratissimum* have been identified by gas chromatography-mass spectrometer (GC-MS) and contained about 92.40% of monoterpenes and sesquiterpenes (Mbakwem-Aniebo *et al*., 2012). Monoterpene are known to contribute to the aroma of the plant (Lexa *et al*., 2007). Fraction of the monoterpenes were characterized by high percentage of eugenol about (68.8%), methyl eugenol (13.21%), cis-ocimene (7.47%), trans-ocimene (0.944%), β-pinene (1.10%) and camphor (0.95%). The therapeutic potentials of the essential oils extracted from fresh leaves of *Ocimum gratissimum* have been discovered to be largely due to the presence of eugenol which is considered as the major

constituents of the essential oil (Lexa *et al*., 2007). The sesquiterpenes were germacrene D (4.25%), trans-caryophyllene (1.69%), α-farnesene (0.85%), β-bisabolene (0.74%) (Rakash and Guptal, 2005).

The chemical constituents of the essential oil of *Ocimum gratissimum* have been previously identified by gas chromatography-mass spectrometery (GC-MS) contained about 92.40% of monoterpenes and sesquiterpenes. The report showed that fraction of monoterpenes were characterized by high percentage of eugenol about (68.8%), methyl eugenol (13.21%), cis–ocimene (7.47%), trans-ocimene (0.944%), β-pinene (1.10%) and camphor (0.95%).

In the present study, GC-MS analysis of the essential oil of *O. gratissimum* revealed twenty- four different compounds comprising of thymol (19.27%), α-phellandrene (12.81%), o-cymene (9.05%), caryophyllene (6.55%), caryophyllene oxide (6.54%), terpinen-4-ol (6.14%) and β-eudesmene (6.01%) as the most predominant. The variation in the percentage composition compared previous findings may be due to geographical factors such as climatic, weather and soil. α-phellendrene, a cyclic monoterpene, has been reported to have anticancer properties inhuman breast cancer cell and human prostate tumor cell (NeseKirimer and Demirci, 2012). It also displayed antimicrobial, antifungal and anti-inflammatory activities (Joshi, 2017).

The palm oil extract of the leaves of *Ocimum gratissimum* (OGPOE) on GC-MS analysis showed it selectively extracted thymol, which may be responsible for the healing property of the extract for cough, wound healing, joint pain, and edema. Thymol has been reported by many authors as a potent antimicrobials agent (Falcone *et al*., 2005). Thymol exhibited anti-inflammatory and antioxidant activities and capable of reducing the risk of cardiovascular diseases, suppressing the progression of hyperlipidemia in high fat-diet rabbit and atherosclerosis by reducing aortic intimal lipids as well as inhibiting oxidative stresses (Mishra and llashukla, 2018). Extensive evidence showed the promising potential of thymol as anti-bacterial and antifungal agents. One of the main uses of palmitic acid on the other hand is in soap because of its ability to help keep skin smooth and also as cleaning agent in certain surfactant (Marchese and Nabavi, 2016).

Fifty seven different compounds were detected by GC-MS upon analysis of the essential oil of *Bryophyllum pinnatum* of which twenty four compounds had been previously reported with the major being nonanal (17.3%) and geranylacetone (13.1%). The most predominant compounds amongst the fifty seven were eucalyptol (11.32%), caryophyllene (10.57%), fenchol (7.17%), patchoulane (6.06%) and spathulenol (3.6%). These compounds have wide range of biological activities. Spathulenol and hexadecylindane were common to the essential oils EO and palm oil extract of the *Bryophyllum pinnatum* (BPPOE).These compounds may be responsible for the healing potential of *Bryophyllum pinnatum* for ear ache. Spathulenol also showed inhibitory capacity for proliferation in the lymphocytes and induced apotosis in cells. Spathulenol is also known to have high antioxidant and antiinflammatory and antimycobacterial properties (Padaha and Verma, 2011).

The result of the volatile oil composition of the leave of *Ocimum gratissimum* by solvent extraction and hydrodistillation revealed other numerous active compounds such as α-thujene, camphene, β-myrlene, thujenol, D-fenchone, linalool, ϒ-terpene, thujene, β-thujene, thujene-2-one, (-) α-caryophyllene, α-panasinsen, naphthalene azulene, linalool, hexyl butanoate, borneol, 1-terpinen-4-ol, thymoquinon, o-terbutylphenol, durenol, verbenone, methyl-9-oxo-nonanoate, α-caryophyllene, eudesma-4(14),11-diene, α-panasinsen, α-cardinol, trans-longipinocarveol, n-hexadecanoic acid, Dehydrodihydrodiisoeugenol (Irian *et al*., 2015). These compounds vary depending on the climate, soil condition and geographical location of the plant (Lexa *et al*., 2007).

Conclusion

There is a global appreciation for use of green solvents in the development of safer chemical processes, manufacturing of food products leading to reduction in environmental hazardous impact, protection of living organisms and sustainable development. *Ocimum gratissimum* and *Bryophyllum pinnatum* are reputable medicinal plants with long of medicinal applications.This piece of work aimed at evaluating palm oil as green extraction medium for bioactive constituents of *O. gratissimum* and *B. pinnatum*. The palm oil extract of the leaf of *O. gratissimum* on GC-MS analysis showed it selectively extracted thymol as the bioactive constituents that may be responsible for the folkloric use of the palm oil extract for healing of wounds, joint pain and edema, whereas the palm oil extract of *B. pinnatum* selectively extracted spathulenol and hexadecylinedane. These compounds may be responsible for the use of *B. pinnatum* for the treatment of severe ear ache.

Conflict of Interest

The authors declare no conflict of interest.

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