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ESSENTIAL OIL COMPOSITION AND BIOACTIVITY OF *THUJA ORIENTALIS* AND *EUCALYPTUS CAMALDULENSIS*

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ABSTRACT

The chemical composition of the essential oil extracted by hydro-distillation using Clevenger apparatus from aerial parts of *Thuja orientalis* and *Eucalyptus camaldulensis* grown in Ondo State, Nigeria was analysed by Gas Chromatography/Mass Spectrometry technique. Toxicity of the essential oils using anti-feedant and filter paper methods on *Callosobrochus maculatus* was also carried out after 6 and 24 hours application. Antibacterial and antifungal activity against ten pathogens which are *Escherichia coli*, *Salmonella paratyphi*, *Bacillus subtillis*, *Staphyclococcus aureus*, *Klebsiella pneumonia*, *Streptococcus pneumonia* as bacteria and *Aspergillus niger*, *Aspergillus flavus*, *Candida tropicallis* and *Fusarium solari* as fungal were carried out. The results of the GC-MS showed that twenty-three compounds representing 93.73% of total oil of *T. orientalis* with α-pinene (32.93%), Δ-3- carene (20.43%), benzyl alcohol (8.69%), myrcene (8.18%), α-terpineol (6.01%) and limonene (5.02%) as the major components. Also, five components which constituted 97.37% of the total oil of *E. camaldulensis* were identified with 1,8-cineole (73.94%), Limonene (9.22%), Cymene (5.90%), Geraniol (5.20%) and α-pinene (3.11%). The results of the toxicity of the Essential oils showed that 100% mortality were recorded for antifeedant of both plants, except at 0.1mL/g concentration for T. *orientalis* which has 96.7% and that of filter paper ranged between 83 to 100% for T. *orientalis* and 70 to 100% for *E. camaldulensis*. T. *orientalis* showed inhibition against all the tested organisms in the order of *Klebsiella pneumonia* > *Bacillus subtillis* > *Streptococcus pneumonia* > *Salmonella paratyphi* > *Escherichia coli* for bacterial and *Candida tropicalis* > *Aspergillus flavus* > *Aspergillus niger* > *Fusarium* solari for fungus. The order of activity of E. *camldulensis* is *staphylococcus aureus* > *Bacillus subtillis* > *Salmonella paratyphi* > *Streptococcus pneumonia* and *Fusarium solari* > *Candida tropicalis* > *Aspergillus flavus* with *Escherichia coli* and *Aspergillus niger* showing resistance to the oil.

Keywords: Essential oils, T*huja orientalis*, Eucalyptus *camaldulensis*, toxicity, bioactivity, organism.

INTRODUCTION

Plants have been used to treat or prevent illness before the advent of synthetic compounds. The sacred Vedas recorded over 5,000 years ago give many references of medicinal plants (Mozhiyarasi and Anuradha, 2016). Plants and plants based compounds are the basis of many of these modern pharmaceuticals we used today for our various ailments (Abraham, 1981). Plant derived bioactive substance are good source of medicines that play a significant role for human health and also used against different types of microbial disease (Ates and Erdogrul, 2003, Sengul *et al*., 2005, Nair *et al*., 2005, Dulger *et al*., 2005, Kumar *et al*., 2006, Mathabe *et al*., 2006). Infections have increased to a great extent and resistant against antibiotics become an ever-increasing therapeutic problem (Jasuja *et al.,* 2013). However, in recent years, plant extract and their phytochemical constituents are getting more importance as they have the great potential sources for microbial growth inhibition and insecticidal activity. A number of researches have focused their interest on investigating the phytochemical constituents of plants for human health (Jasuja *et al*., 2012).

For the control of insects in stored grains, synthetic chemical products belonging to different toxicological classes are commonly used. However, concerns regarding the continuous use of these conventional insecticides, including difficulties in registration of those insecticides in some countries and development of resistance by pest organisms, persistence in the environment, elimination of beneficial organisms, mammalian toxicity, residues on food, higher cost of crop production and technical difficulties at times of application have led researchers to evaluating new reduced-risk insecticides to control stored-product pests protection (Daglish, 2004, Sousa *et al*., 2009).

The present study seeks to determine the chemical composition and the bioactivity (the insecticidal, antibacterial and antifungal activity) of *T. orientalis*

and *E. camaldulensis* against some pathogens and cowpea bruchid (*Callosobrochus maculatus*).

MATERIALS AND METHODS:

Plant Materials

The aerial parts of *T. orientalis* and *E. camaldulensis* were collected in month of September, 2016 at the premises of AdekunleAjasin University, Akungba-Akoko, Ondo State of Nigeria. The plants were authenticated in the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba- Akoko, Ondo State.

Isolation of the volatile oil

Fresh aerial parts of the plants were washed free of sand and other impurities and cut into small pieces. Five hundred grams was hydrodistilled for two hours using a Clevenger apparatus. The oils were dried over anhydrous Na_2SO_4 and kept in a sealed sample bottle at 4°C until analysis.

Gas Chromatography/Mass Spectrometry Analysis

The analysis of the volatile compounds was carried out on a Hewlet Packard 6890 GC/MS system equipped with quartz capillary column; 30m x 0.25mn i.d x 0.25µm film thickness. The carrier gas was helium (1ml/min); oven temperature, 40°C to 300°C at a rate 5°C/min then held isothermal for 2min. The injector port temperature was 250°C. The ionization of the sample components was performed on E.I mode (70ev). The identification of different constituents was performed by comparison of their retention time and mass spectra with those of the library.

Insect rearing and maintenance

The initial stock of cowpea bruchid (C. *maculatus* (F)) used for the study was obtained from an already infested cowpea seeds purchased from a local market, Okusa Food Market in Akungba-Akoko, Ondo State of Nigeria in February, 2017. From this stock, new generation was reared in laboratory in on cowpea at room temperature. Freshly emerged adults of C. *maculatus* were then subsequently sub-cultured on the same variety of cowpea over four generations before they were used for experiment.

Antifeedant Test

Four concentrations of *T. orientalis* and *E. camaldulensis* oils; 0.01, 0.02, 0.03, and 0.04mL were dissolved separately in 0.5 mL of acetone. Each of the concentrations for each oil was admixed with 10g of cowpea contained in 50 ml glass jar. The admixture was stirred thoroughly with a glass rod to ensure adequate coating of seeds with oil and until the acetone completely evaporated according to the method of Lale (1991). Twenty mixed sex adults of C. *maculatus* (3-5 days old) were introduced into each jar and the lid was replaced. Control seeds were treated with 0.5mL pure acetone only and second control was only cowpea without any treatment. Each treatment and control were replicated three times. Mortality record was taken at six and twenty-four hours interval after introducing insects on the seeds. Insects which did not respond to the gentle touch of a small probe were considered death (Su, 1976)

Filter paper test

Bioassay on the toxicity of *T. orientalis* and *E. camaldulensis* essential oils against adult *C. maculates* was similar to the method described in Ukeh *et al.,* 2012) in Pyrex glass Petri dishes (10 cm diameter). Different doses of each essential oil (0.01, 0.02, 0.03, and 0.04mL) were dissolved in 0.5 mL acetone and delivered to the Petri dishes pre-lined with Whatman Number one filter paper. Pure acetone was used for the filter paper for control. The solvent was allowed to evaporate and then twenty mixed sex of *C. maculates* adults were introduced into each Petri dish. The Petri dishes were closed and maintained in the laboratory for 6 and 24hrs at ambient temperature and 55 – 67 Relative Humidity. All treatments were replicated three times for each dose of all essential oils, and record of dead weevils was taken at 6 hours and 24 hours interval

Antimicrobial activity of essential oils

The micro-organism used in this study were isolates collected from out patients ward of the Federal Medical Centre, Owo whose morphological and biochemical characteristics were confirmed. The bacterial cultures were maintained on nutrient broth while the fungal cultures were maintained on sabouraud liquid medium. The bacteria and fungi used includes three gram negative bacteria *Escherichia coli,*

Klebsiella pneumonia and Salmonella paratyphi, three gram positive bacteria *Bacillus subtillis, Staphylococcus aureus, and Streptococcus pneumonia;* and four strains of fungi; *Aspergillus flavus, Candida tropicalis, Fusarium solani* and *Aspergillus niger*.

Zone of inhibition

Inoculums size containing 10 cfu/mL for bacterial and 10 sfu/mL for fungal were used to seed already solidified Petri plates of Muller-Hinton agar. The antimicrobial activities of the oil were determined using agar well diffusion method. Ten organisms were used in all three gram positive,three gram negative and four fungi. A sterile 6mm cork-borer was used to make well on already solidified agar, the wells were filled with the oil ensuring that they were allowed to stand for about 2 hours to allow absorption of the oil into the medium after which they were incubated at 37°C for fungi for 24 hours for bacterial and 7 days for fungi.

Minimum inhibitory concentration (MIC)

A modified Macro-broth dilution technique was used in this research for MIC. Those recorded as MIC were the lowest concentration of the tested oil that showed no visible growth of the tested isolate. Serial dilutions of the oil were carried out to give a concentration of 0.5mL/mL, 0.25mL/mL, 0.125mL/mL, 0.0625mL/mL. 2mLof each diluted concentration was added to 18ml of pre-sterilized molten Mueller-hinton and Sabouraud agar mixed properly and allowed to set. After which the standardized inoculums were seeded on the plates. The bacterial plates were incubated at 37°C for 24 hours, while the fungi at 25°C for 7 days. The results were observed and recorded.

RESULTS AND DISCUSSION

The essential oil obtained from the aerial parts of *T. orientalis* and *E. camaldulensis* when subjected to GC-MC analysis showed twenty-three (23) components representing 94.08% of the total composition of *T. orientalis* oil and five (5) components representing 97.37% of the total composition of *E. camaldulensis*, as showed in the Tables 1 and 2, respectively. The results for *T. orientalis* revealed the presence of 79.68% monoterpenes (70.97% monoterpene hydrocarbons and 8.71% oxygeneated monoterpenes) 5.4% sesquiterpenes (4.70% sesquiterpene hydrocarbons and 0.70% oxygenated sesquiterpenes), 8.69% benzyl alcohol and 0.31% tetra decanoic acid.

Furthermore, Table 1 shows that α-pinene (32.93%), Δ-3- Carene (20.43%), Benzyl alcohol (8.69%), Myrcene (8.18%), α-terpineol (6.01%), Limonene (5.02%), Camphene (2.89%), Borneol acetate (2.01%), α-Humulene (12.7%) and Germacrene D. (1.14%), were the main constituents with α-pinene (32.93%) the most predominant among other constituents. Nickavar *et al*., 2003 reported that *T. orientalis* cultivated in Iran has α-pinene (21.90%), Δ-3- carene (10.50%) and limonene (7.20%) as the main constituents.

The result from Table 2 showed that *E. camaldulensis* was dominated by monoterpenoids (97.37%) with (18.23% monoterpene hydrocarbons and 79.14% oxygenated monoterpenes). The major constituents of *E. camaldulensis* are 1,8-cineole (73.94%), Limonene (9.22%), Cymene (5.90%), Geraniol (5.20%) and α-pinene (3.11%). Basak and candan (2010) have reported the major components of leaves oil of *E. camaldulensis* found in Iran to be P-cymene (68.43%) 1,8-cineole (13.92%), α-pinene (3.45%) and Limonene (2.84%). Sliti *et al*., 2015, in their study compared the chemical composition of two species of *Eucalyptus* lead oil (*E. camaldulensis* and *E. riduis*) from Korbous (Tunisa) and in their study, it was reported that the major compounds found in the leaf oil of *E. camaldulensis* were Spathulenol (20.2%), P-cymene (14.83%), 1,8-cineole (12.16%), Phellandral (6.6%), Cryptone (7.02%), Globulol (6.16%) and Terpen-4-o1 (5.25%) and for *E.rudius*, it was with spathulenol (17.47%), P-cymene (20.49%), 1,8-cineole (14.61%), Cryptone (10.38%), phellandral (4.55%) and Terpen-4-o1 (5.25%) being the dominant ones. Moreover, Sliti *et al*., 2015 and Cheng *et al*., (2009), reported high amounts of α-phellandrene, P-cymene, α-pinene, 1,8-cineole, γ-terpinene in *E. camaldulensis* oils extracted from Taiwan. It would be noteworthy to point out that the composition of any plant essential oil studied is influence by the presence of several factors, such as local, seasonal variation and experimental conditions (Olonisakin, 2010).

Table 1: Chemical Composition (%) of Thuja orientalis essential oil

S/N	Compound Name	Retention Time	Composition (%)
1	Benzyl Alcohol	3.62	8.69
2	Camphene	5.98	2.89
3	Limonene	7.79	5.02
4	α-Pinene	9.82	32.93
5	β-Pinene	10.67	0.43
6	Δ-3- carene	11.36	20.43
7	Trans ocimene	12.24	0.32
8	Cis Ocimene	12.90	0.77
9	Myrcene	12.99	8.18
10	Camphor	15.02	0.38
11	Neral	15.29	0.31
12	α-terpineol	18.67	6.01
13	Borneol Acetate	21.60	2.01
14	β-bisabolene	21.85	0.33
15	β-caryophyllene	22.42	0.85
16	α-Humulene	22.56	1.27
17	α-Bergamotene	22.68	0.34
18	α- Farnesene	22.89	0.33
19	Gama Cadiene	22.94	0.44
20	Germacrene D.	24.02	1.14
21	Spathulenol	26.72	0.35
22	Caryophyllene Oxide	27.06	0.35
23	Tetra Decanoic Acid	30.53	0.31

Table 2: Chemical Composition (%) of Eucalyptus camaldulensis essential oil

S/N	*Compound Name*	*Retention Time*	*Composition (%)*
1	Cymene	6.96	5.90
2	Limonene	9.08	9.22
3	α-Pinene	9.83	3.11
4	Geraniol	14.86	5.20
5	1,8-cineole	16.60	73.94

Table 3 revealed that 100% mortality were recorded in all the concentration used at 6 and 24 hours duration, except for concentration of 0.1mL/g of T. *orientalis* that had 96.7% 00%. Results obtained so far showed that EOs of both herbal plants are toxic to *C. maculatus* by inhibiting the feeding and survival of the weevils. Previous results obtained by Mohammed and Abdelgaleil (2008) showed that essential oil of *E.camadulensis* was potent against *S. oryzae* and *T. castaneum*. Tunc *et al.*, 2000, also reported that oil of *E. camaldulensis* shows 45 percent mortality of eggs of *T. confusum* and *E. kuhnie*lla. It has also been reported that major components of oil when in combination with other compounds of diverse structure in the oil could exhibit different mode of action against organism (Jilani and Su, 1983).

Table 3: Results of the Antifeedant Test of T. *orientalis* against C. *maculatus*

Mean mortality (%) ± SD

	T. orientalis		*E. camaldulensis*	
Conc. (mL/g)	**6hrs**	**24hrs**	**6hrs**	**24hrs**
0.01	96.7 ± 0.3[b]	100.0 ± 0.0[a]	100 ± 0.0[a]	100 ± 0.0[a]
0.02	100.0 ± 0.0[a]	100.0 ± 0.0[a]	100 ± 0.0[a]	100 ± 0.0[a]
0.03	100.0 ± 1.2[b]	100.0 ± 00[a]	100 ± 0.0[a]	100 ± 00[a]
0.04	100.0 ± 0.0[a]	100.0 ± 0.0[a]	100 ± 0.0[a]	100 ± 0.0[a]
P	0.001	0.001	0.001	0.001
LSD (0.05)	-	-	-	-

Table 4: Results of the filter paper test of T. *orientalis* against C. *maculatus*

Mean mortality % ± SD

	T. orientalis		*E. camaldulensis*	
Conc. (vmL/cm^2)	**6hrs**	**24hrs**	**6hrs**	**24hrs**
0.01	83.3 ± 0.3[b]	90.0 ± 0.9[a]	70 ± 0.6[b]	100 ± 0.0[a]
0.02	86.0 ± 1.0[b]	93.0 ± 0.9[a]	73.3 ± 0.9[b]	100 ± 0.0[a]
0.03	90.2 ± 0.6[a]	98.0 ± 1.0[a]	76.0 ± 0.3[a]	100 ± 0.0[a]
0.04	96.0 ± 1.5[a]	100.0 ± 0.3[a]	82.0 ± 0.3[a]	100 ± 0.0[a]
P	0.001	0.001	0.001	0.001
LSD (0.05)	13.00	2.00	2.0	-

Results from Table 4 showed that both oils enhance the mortality of C. *maculatus* with an increased mortality rate as the concentration increases. The mortality rate of *C. maculatus* to *E. camaldulensis* was higher than *T. orientalis* after 6 and 24 hours which may be as a result of 1,8-cineole (73.94%), an active ingredient present in *E. camaldulensis* as reported by Batish *et al*., 2008. Mohamed and Abdelgaleil (2008) has also reported that *E. camaldulensis* exhibited a good toxicity against *S. oryzae* in fumigation bioassay test.

Results from Table 5 and 6 showed the antimicrobial activity of *T. orientalis* and *E. camaldulensis* essential oils against ten pathogens (Six bacteria and four fungus). The result revealed that the essential oils inhibited the growth of test organisms at varying degrees. *T .orientalis* was shown to be effective on all the test organisms in the order of *Klebsiella pneumonia* (1.73mm) > *Bachillus subtillis* (.160mm) > *Streptococcus pneumonia*(1.50mm) > *Salmonella paratyphi* (1.40mm) > *Escherichia coli* (1.33mm) and *Candida tropicalis* (1.60mm) > *Aspergillus flavus* (1.40mm) > *Aspergillus niger* (1.33mm) > *Fusarium solari* (1.20mm).

For bactericidal and fungal activity, respectively. *Echerichia coli* and *Aspergillus niger* were resistant to E. *camaldulensis* oil but showed high activity against two bacterial and two fungal in the order of *Staphylococcus aureus* and *Fusarium solari* (1.73mm) and *Bacillus subtillis* and *Candida tropicallis* (1.63mm). The MIC results from Table 6 showed that at the highest concentration of 0.5mL/mL, the oils inhibited the growth of all the organisms used except the oil of H. *suaveolen* that could not suppress E. *coli.*

There was no inhibition of the pathogens at concentration of 0.0625mL/mL for all the oils. Baser *et al*., (2002) and Sivropoulou *et al*., (1997) had earlier reported that high antibacterial and antifungual activity of *T. orientalis* were due to high contents of α-and β-thujone which is the most active component of the oil grown in Rajasthan area of India.

Antimicrobial activity:

Table 5: zone of inhibition (mm) of essential oil extracted from *T. orientalis* and *E. camaldulensis.*

Name of Organism		**Negative Control (Distilled Water)**	**Positive Control (Chloramphenicol**
Escherichia Coli			
T. Orientalis	1.33	0.00	2.00
E. Camaldulensis	0.00	0.00	2.20
Salmonella Paratyphi			
T. Orientalis	1.40	0.00	3.00
E. Camaldulensis	1.36	0.00	3.00
Bacillus Subtillis			
T. Orientalis	1.60	0.00	3.80
E. Camaldulensis	1.63	0.00	3.20
Staphylococus aureus			
T. Orientalis	1.20	0.00	3.50
E. Camaldulensis	1.73	0.00	3.00
Klebsiella Pneumonia			
T. Orientalis	1.73	0.00	2.50
E. Camaldulensis	1.16	0.00	3.00
Streptococcus Pneumonia			
T. Orientalis	1.50	0.00	3.50
E. Camaldulensis	0.23	0.00	2.70
Aspergillusniger			
T. Orientalis	1.33	0.00	2.00
E. Camaldulensis	0.00	0.00	2.20
Aspergillus flavus			
T. Orientalis	1.40	0.00	3.00
E. Camaldulensis	1.36	0.00	3.00
Candidatropicalis			
T. Orientalis	1.60	0.00	3.80
E. Camaldulensis	1.63	0.00	3.20
Fusarium Solari			
T. Orientalis	1.20	0.00	3.50
E. Camaldulensis	1.73	0.00	3.00

Table 6: Minimum Inhibition Concentration (MIC) mL/mm of *T. orientalis* and *E. camaldulensis*

Name of Organism	0.5	0.25	0.125	0.0625
Escherichia Col.				
T. Orientalis	+	+	-	-
E. Camaldulensis	-	-	-	-
Salmonella Paratyphi				
T. Orientalis	+	+	-	-
E. Camaldulensis	+	+	-	-
Bacillus Subtillis				
T. Orientalis	+	+	-	-
E. Camaldulensis	+	+	+	-
Staphylococcus aureus				
T. Orientalis	+	+	-	-
E. Camaldulensis	+	+	+	-
Klebsiellapneumonia				
T. Orientalis	+	+	+	-
E. Camaldulensis	+	+	-	-
Streptococcus Pneomonia				
T. Orientalis	+	+	+	-
E. Camaldulensis	+	+	+	-
Aspergirrusniger				
T. Orientalis	+	+	+	-
E. Camaldulensis	+	+	+	-
Asperginusflavus				
T. Orientalis	+	+	+	-
E. Camaldulensis	+	+	+	-
Candida tropicalis				
T. Orientalis	+	-	-	-
E. Camaldulensis	+	+	-	-
Fusariumsolani				
T. orientalis	+	+	+	-
E. camaldulensis	+	+	+	-

+ indicates positive response; - indicates negative response.

According to Sliti *et al*., 2015, in their study with two species of *eucalyptus* leaves (*E. camaldulensis* and *E. rudis*), the antibacterial activity of the essential oils against some bacterial can be attributed to the presence of (+) – spathulenol present in the greatest proportion and P-cymene, which represented 14.83-49% of the essential oils tested. The activity of *T. orientalis* in this study to some of the pathogens may be as a result of its high concentrations of α-pinene (32.93) and 3-carene (20.43) and that of *E. camaldulensis* may be as a result of 1,8 cineole (73.94) which are the most predominant compounds in the oils.

CONCLUSION

α-pinene (32.93) and 1,8- cineole (73.94) has been found be the major components of *T. orientatlis* and *E. camaldulensis,* respectively in Akoko, Ondo State, Nigera. The oils toxicity (antifeedat and filter paper) test were found to be concentration

dependent. The present results showed that the essential oils from these plants have the potential to be used in pest control for storage grain/seeds. The oils were also found to inhibit some pathogenic organisms and hence could find application in medicinal industry. Pathogens are economically damaging human and animal health, agriculture and food. Thus, the use of natural products like essential oils from *T. orientalis* and *E. camaldulensis* may lead to economic benefit to the grown population in the world.

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