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Effect of harvest time on essential oil composition of *Chromolaena odorota* (L.) leaves from Nigeria .

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Abstract:
Essential oils were obtained from fresh leaves of *Chromolaena odorota* (L.) harvested at different times of the day (morning and afternoon) by hydro distillation using the Clevenger apparatus. The oils were analyzed by Gas Chromatography (GC) and Gas Chromatography – Mass Spectrometry (GC-MS). Caryophyllene (6.40%) and aromadendrene (5.56%) were the major sesquiterpenoids in the morning sample while α- pinene (9.09%) and β-pinene (5.10%) were the main monoterpenoids detected. The principal sesquiterpenoids in the leaf oil of the afternoon sample are Germacrene D (4.70%), aromadendrene , α-amorphene and ϒ-muuolene which were detected in the same amount of 4.12% while the main monoterpenoids detected are α-pinene (5.63%) and β-pinene (4.83%).
Key words: *Chromolaena odorata*, GC-MS, aromadendrene, terpenes, asteraceae

Introduction:
Chromolaena odorata (L.) is one of the world's tropical weeds. It is a member of the tribe *Eupatirieae* in the sunflower (Asteraceae) family. It is native to Central and South America but is now distributed throughout Africa and tropical Asia, extending from West, Central and Southern Africa to India, Sri Lanka, Bangladesh, Laos, Cambodia, Thailand, Southern China, Taiwan, Indonesia etc. (Bani, 2002; Muniappan and Marutani, 1991; Chomnawang *et al*., 2005; Umukoro et al., 2006). It goes by different common names including Siam weed, devil weed, French weed, communist weed, hagonoy and cohoy among others. *Chromolaena odorota* is an important medicinal plant with the extracts of its' fresh leaves being used in Ghana and Benin for the treatment of malaria (Ayensu et al., 1978). In Ivory Coast, it is used as cataplasms to stop haemorrhage and as an anti inflammatory drug against pain (Bedi *et al*., 2001).The plant decoction is used as a remedy for coughs and colds and to treat wounds, skin infections and inflammation (Adjanohoun & Ake – Assi, 1979; Inya-Agha *et al*., 1987; Owolabi *et al*., 2010; Apichart *et al*., 2004; Rajesh, 2013). It is also used as an antiplasmodic, antiprotozoal, antitrypanosomal, antibacterial, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic and hepatotropic agent (Igboh *et al*., 2009). A number of studies have been carried out to validate the medicinal uses of this plant, studies have shown that the leaf extract has anti oxidant, anti-inflammatory, analgesic, anti – microbial, cytoprotective and many other medicinally significant properties (Warea, 2004; Ling et al., 2007;)
The essential oil of the plant has also been shown to exhibit insecticidal, insect repellent and antibacterial activities (Owolabi *et al*., 2010)
Phytochemical studies on the leaves extract showed the presence of tannins, steroids, flavonoids, cardiac glycosides and alkaloids (Ahmad et al., 1967; Hai et al., 1995; Akinmoladun et al., 2007; Igboh et al., 2009).
The essential oil from this plant from different parts of the world have been studied extensively (Owolabi *et al*., 2010; Bedi *et al*., 2010; Avlessi *etal*., 2012), however there have been wide variations in the composition of the essential oil of this plant reported in literature attributed to possible differences in growing conditions or chemotype (Velliagiri et al., 2011). However, variations have also been noticed even in samples obtained from same environment (Inya – Agba et al., 1987; Owolabi *et al*., 2010). Thus in this study, we investigated the effect of time of collection on composition and antimicrobial activity of the essential oil from the leaves of *Chromoleana odorota* .

Experimental
Plant Collection: Fresh leaves of *Chromolaena odorota* was collected in the morning and in the afternoon within the premises of Ladoke Akintola University of Technology, Ogbomoso, Oyo state, Nigeria and identified at the Department of Agronomy of the same University.
Oil Isolation: 500 g of the fresh leaves were hydrodistilled for 4 hrs using the Clevenger apparatus and the oil extracted into hexane to avoid hydrosorption. The oil was later collected into sample bottle, sealed and stored

under refrigeration until time of analysis. The essential oil for the afternoon sample was obtained in a similar manner.

Characterization: GC-MS analysis of the essential oils were performed using a Shimadzu Gas Chromatograph Model QP2010 plus, a gas chromatographic (GC) system, equipped with a Mass selective detector and auto injector. Compounds were separated on capillary column (30 m x 0.25 mm, film thickness 0.25 µm). 1.0 µl of sample was injected using the split mode (split ratio 1: 100). For GC/MS detection , an electron ionization system with ionization energy of 70 eV was used. Column oven temperature program was the same as previously used in GC analysis. Helium was used as a carrier gas at a flow rate of 1.5 $mlmin^{-1}$. Mass scanning range was 40-700 m/z while injector and MS transfer line temperatures were set at 220 and 290^{o}C, respectively.

Results and discussion

Table 1: Composition of the essential oils in morning and afternoon samples of *Chromolaena odorata*

SN	COMPOUNDS	RETENTION INDEX	%COMPOSITION MORNING SAMPLE	%COMPOSITION AFTERNOON SAMPLE
1	3- Methylhexane	653	3.09	-
2	2- Ethylpentane	653	-	3.54
3	n-Heptane	717	12.36	14.16
4	Tetrahydrofuran	723	1.20	1.00
5	Cyclopentane	733	4.04	4.49
6	1,3-cycloheptadiene	804	4.04	4.49
7	2-Hexenal	814	3.25	2.83
8	Oxetanol	815	1.20	-
9	3-Bromohexane	850	1.20	1.00
10	2- Hexanone	868	1.20	1.00
11	Sabinene	897	-	1.12
12	Nitrohexane	935	1.20	1.00
13	Bicyclo[3.1.1]hept-2-ene	943	3.03	-
14	Beta-pinene	943	5.10	4.83
15	Alpha-pinene	948	9.09	5.63
16	Ocimene	958	1.26	0.66
17	Beta-ocimene	976	1.89	2.62
18	6-Propenylbicyclo[3.1.0]hexan-2-one	1061	4.04	4.49
19	3,4-diethenyl-3-methylcyclohexene	1076	4.04	4.49
20	5,6-diethenyl-1-methylcyclohexene	1092	4.04	4.49
21	Cyclopropane	1115	3.96	-
22	Copaene	1221	0.47	1.16
23	2 – cyclopropylidene – 1,7,7 – trimethylheptane	1251	-	4.12
24	Cubebene	1344	0.64	-
25	Aromadendrene	1386	5.56	4.12
26	Gamma-muurolene	1435	3.96	4.12
27	Alpha-amorphene	1440	3.96	4.12
28	Delta-cadinene	1469	0.47	0.58
29	Caryophyllene	1494	6.40	-
30	Bicyclo [7.2.0]undec – 4 – ene	1494	-	6.76
31	Germacrene D	1515	0.79	4.70
		TOTAL	91.48	92.52

Table 1 shows the volatile compounds identified in the essential oil obtained from *Chromolaena odorota* leaf samples harvested in the morning and in the afternoon. Twenty seven compounds were identified in the oil from the sample obtained in the morning constituting 91.48% of the oil while a total of twenty five compounds were identified in the afternoon sample constituting 92.52% of the oil. Hydrocarbons are the most abundant components in the two oil samples, constituting 38.6% of the morning sample and 46.54% of the afternoon sample. This is in agreement with literature reports in which the hydrocarbons constitute the major constituents of the leaf essential oil of *Chromalena odorota* (Avlessi et al., 2012). Sesquiterpenoids constitute 22.25% of the morning sample and 18.8% of the afternoon sample. The monoterpenoids were detected at 17.34% in the morning sample while they constitute 14.86% of the afternoon sample. The oxygenated hydrocarbons were found to constitute 10.89% of the morning sample and 10.32% of the afternoon sample.

Disregarding the n-hexane and n-heptane constituents of the oils which are obviously from the residual hexane used in the extraction of the oils, the major constituents in the essential oil of the leaf sample collected in the morning are α – pinene 9.09%, caryophyllene 6.40%, aromadendrene 5.56% and β – pinene 5.10%. Cyclopentane, 1,3- cycloheptadiene, 6 – propenylbicyclo [3.1.0] hexan – 2 – one, 3,4 – diethenyl – 3 – methylcyclohexene and 5,6 – diethenyl – 1 – methylcyclohene were each detected at 4.04% in the oil. The major constituents in the afternoon sample are: Bicyclo [7.2.0] undec – 4 – ene 6.76%, α – pinene 5.63%, β – pinene 4.83%, germacrene D 4.70% and aromadendrene 4.12%. Cyclopentane, 1,3 – cycloheptadiene, 6 – propenylbicyclo [3.1.0]hexan-2-one, 3-Methyl-3,4-divinyl-1-cyclohexane and 1-Methyl -5,6-divinyl-1-cyclohexene were each detected in the afternoon oil at 4.49%. This shows that there is a substantial difference in the composition of the two oil samples, caryophyllene 6.40% which was a major constituent of the morning sample was not detected at all in the afternoon sample. In cases where the major constituents in both the morning and afternoon samples are the same, the proportions of these compounds are different in the two oils. It is interesting to note that Cyclopentane, 1,3- cycloheptadiene, 6 – propenylbicyclo [3.1.0] hexan – 2 – one, 3,4 – diethenyl – 3 – methylcyclohexene and 5,6 – diethenyl – 1 – methylcyclohene were each detected in equal amount of 4.04% in morning sample and 4.49% in the afternoon sample.

The major compounds that have previously been identified from essential oil of *C.odorata* are α-pinene 21.15%, geigerene 11.68% and pregeigerene 19.61% (Bedi *et al*., 2001) from Ivory coast; β-caryophyllene 21% and germacrene D 15.3% (Sohounhloue *et al*., 1996); Bicyclogermacrene 12.55%, geigerene 11.85%, (Z)-β-farnesene 9.98% and α – pinene 9.36% (Pamo et al. 2004); pregeijerene 19.9%,α-pinene 17.9%, β-caryophyllene 21.0% and germacrene – D 15.3% (Noudogbesi et al., 2006;); α-pinene 20.7%, pregeijerene 14.6%, geijerene 12.0%, β –pinene 10.3% and D – germacrene 9.7% (Avlessi *et al*., 2012) from Benin; α-pinene 20.7%, geijerene 3.1% and pregeijerene 17.6% from Thailand (Pissuthanan *et al*., 2006); trans – caryophyllene 16.6% from China (Ling *et al*., 2003) and ascaridole 51.1% from Togo (Kofi *et al*., 2009).

It is interesting to note that the composition of the essential oil of the two samples is somehow different from literature reports on essential oil from this plant even from Nigeria. For example limonene, camphor, cardinal, geijerene and pregeijerene were reportedly present in the essential oil of *Chromolaena odorota* leaf from Nigeria (Inya – Agba et al., 1987), they were not detected from our samples. Similarly, in another study on composition of leaf essential oil of *Chromolaena odorota* collected from Epe, Nigeria, (Owolabi *et al*., 2010) α-pinene (42.2%), β-pinene (10.6%), germacrene D (9.7%), β-copaen-4α-ol (9.4%), (E)- caryophyllene (5.4%) and geijerene/pregeijerene (7.5%) were the main compounds identified in the essential oil. Although, most of these compounds except for β-copaen-4α-ol and geijerene/pregeijerene are also detected in our samples, they were found in very small amounts. It is also interesting that 5,6-diethenyl-1-methylcyclohexene constituting 4.04 and 4.4 9% of the morning and afternoon samples respectively has not been previously reported from *Chromolaena odorata* from Nigeria, however, it was the major constituent (44.7%) of *C. odorota* essential oil from India (Velliangiri *et al*., 2011). Interestingly too, as in this study, geijerene and pregeijerene were not detected in the Indian sample, suggesting that they may be of the same chemotype.

There were six compounds that were detected at different concentrations in the leaf oil of the morning harvest but which were not detected at all in the afternoon sample. These are Caryophyllene (6.40%), 3 – methylhexane

(3.09%), cyclopropane (3.96%), Bicyclo[3.1.1]hept-2-ene (3.03%), Cubebene (0.64%) and oxetanol 1.20%. Similarly, four compounds were found in the afternoon oil but were absent in the morning sample, these are 2 – Ethylpentane (3.54%), Sabinene (1.12%), Bicyclo [7.2.0]undec – 4 – ene (6.76%) and 2 – cyclopropylidene – 1,7,7 – trimethylheptane (4.12%)

. The variation in the constituents of the oil samples in the leaf harvested in the morning and the leaf harvested in the afternoon could be attributed to light effect since all other conditions are the same. While some of the components may be heat labile and are therefore possibly lost to heat from the sun hence their absence in the afternoon sample, formation of some compounds may have been enhanced in the presence of heat /light and this probably account for their presence in the afternoon sample but absence in the morning sample.

Conclusion:

This study has shown that time of harvest may have effect on the composition of leaf essential oil of *Chromolaena odorota*, with the morning sample showing more constituents and higher proportion of the common constituents than the afternoonsample.

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