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COMPARATIVE STUDY OF CHEMICAL COMPOSITION OF *ARTEMISIA ANNUA* ESSENTIAL OIL GROWING WILD IN WESTERN CAMEROON AND LUXEMBOURG BY µ-CTE/TD/GC/MS

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ABSTRACT:

The composition of essential oils of Artemisia annua from seven localities of West Cameroon and from Luxembourg were determined. The essential oils were extracted by micro-chamber thermal extractor (µ-CTE) and analyzed by Thermal Desorption plus Gas Chromatography coupled to Mass Spectrum (TD/GC/MS). According to the results obtained in this study, artemisia ketone was present only in the samples from Luxembourg. Limonene, eucalyptol and copaene were also found to be present in higher concentration in the Luxembourg samples while various localities of Cameroon were found to be richer in camphor and menthol. Camphor was the major compound among the thirteen identified and quantified from the extracts of both countries even though its yield was>60% in the Cameroon samples and 35.67% in that of Luxembourg. Climatic and culture conditions could be responsible for the variation in chemical composition of the samples studied. This work was carried out in an accredited laboratory (ISO 17025) by using validated method.

Keywords: ***Artemisia annua, Western Cameroon, essential oil, µ-CTE, TD/CG/MS, camphor.***

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1. INTRODUCTION

A. annua (Asteraceae) is an aromatic, annual and perennial plant up to 1-3 m high and 1 m wide (Delabrays *et al*., 1992). This species was introduced in Cameroon where their teas are been used for the treatment of malaria. The cultivation of this plant in many regions of Cameroon (West, North-West and South-West) is done under the control of some organizations such as CIPCRE (International Circle for the Promotion and Creation) which provides the high quality seeds from MEDIPLANT (Research Center of aromatic plants Conthey-Switzerland).

Previous studies on this plant showed that it has anti-inflammatory, analgesic, antiseptic, antiviral and anticancer activities (Pierre, 2009). These pharmacological activities are due to the presence of certain chemical constituents such as terpenoids, coumarins, flavonoids, polyphenols and volatile compounds (Ferreira*et al.*,2010), (Verdian-Rizi*et al.*,2008). The essential oil from the Artemisia species was used in ancient Greece and in the Roman Empire; in infusion as poison antidote and for its abortive qualities indicated in gastric insufficiency. It has also been reported to possess antimalaric and antihelmintic properties and the ability to activate blood circulation (Ferreira*et al.*,2010).

Variations in the chemical composition and thus biological activities of*Artemisia annua* essential oils from species growing in different geographic locations including Bulgaria and India(Verdian-Rizi*et al.*,2008), (Bhakuni*et al.*, 2001), (Woerdenbag *et al.*, 1993), (Tzenkova, *et al.*, 2010) have been reported. These differences have been reported to be due to factors such as climatic and culture conditions, chemical and biological treatments, drying temperature, etc. (Verdian-Rizi*et al.*,2008). The aim of this study was therefore to compare the chemical composition of essential oils of *Artemisia annua* from Western Cameroon - a tropical region and Luxembourg - a temperate region.

2. EXPERIMENTAL SECTION

2.1. Plant material

The leaves of *Artemisia annua* were collected before the flowering period from the farmer's plantations in the Grassfield Regions of Cameroon from June to December, 2009, between 9 a.m. and 3 p.m (Delabays*et al.*, 1993), (Ferreira and Janick, 1996). The localities of collection were Bangang-Fokam, Bangangte, Bandjoun, Bafoussam (CIPCRE), Mbouda, Dschang, Bamenda (West Cameroon) and Walferdange (Luxembourg). The samples were identified by a botanist of the National Herbarium of Cameroon, Yaounde, where a voucher specimen (No. 65647 HNC/Cam) was deposited.

2.2 Extraction by µ-CTE

The leaves of *Artemisia annua* from various localities were separately dried under a shaded and well ventilated place. The dried leaves were crushed with an automatic electric crusher (Retsch MM400®) to obtain a dried powder.

10 mg of crushed sample was introduced into a chamber or cell, and a controlled flow of air passed through all chambers simultaneously. After an equilibration period (typically 20-30 minutes), conditioned sorbent tubes (with 200 mg of Tenax ™) were attached to each micro-chamber to begin the vapour sampling process (Schripp*et al.*, 2007), (Williams and Pharaoh, 2009). As the pure air passes over the surface or around the bulk sample with 20 mL/min flow, vapours were swept from the material, out of the micro-chamber onto the attached sorbent tube at 100°C during a period of one hour (Schripp*et al.*, 2007), (Williams and Pharaoh 2009). These sorbent tubes were desorbed with a thermal desorption unit (Unity Thermal Desorption, Markes ™) and then analyzed with GC/MS (Schripp*et al.*, 2007), (Williams and Pharaoh, 2009).

2.3. Analysis by TD/CG/MS

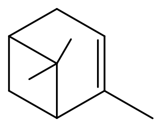
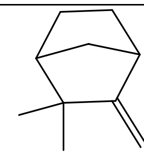
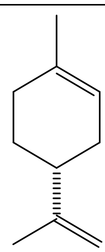
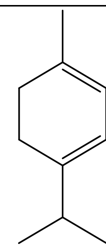
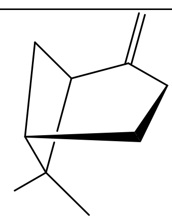
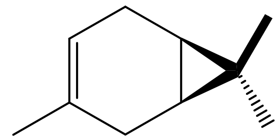
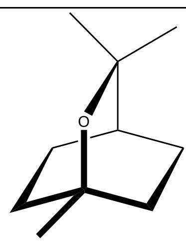
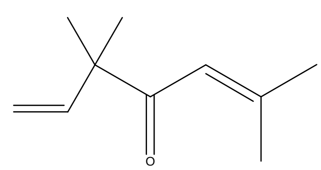
The identification and quantification of the essential oils from the extracts were carried out with Gas Chromatography 6890 Network GC System ND of Agilent Technologies, 5973 Inert Mass Selective Detector of Agilent Technologies with Unity Thermal Desorber of Markes™. Oven was programmed from 60°C (5 min) to 240°C at 8°C/min and held for 7.5 min with capillary column (HP-Innowax, 60 m length x 0.32 mm internal diameter and 0.5 µm film thickness). Injector (Markes Unity TD) and Flame Ionization Detector temperatures were 70°C and 140°C respectively. Helium was used as carrier gas constant column pressure 11.7 psi (flow at start temperature 1.5 mL/min and drops with oven heating program).

Mass spectral data were acquired in the scan mode in the *m/z* range 40-350 (Woerdenbag *et al.*, 1993), (Wei *et al.*, 2004). The essential oils constituents were identified by matching their mass spectra and retention indices (RI) with those of reference compounds. Standards used were α-pinene (p.a), β-pinene (p.a), 3-carene (p.a), limonene (p.a), camphene (95 % pure), camphor (99 % pure), (trans-)caryophyllene (98.5 % pure), copaene (90 % pure), eucalyptol (99 % pure), menthol (100 g, 98 %), α-terpinene (purum), α-terpineol (97 % pure) and artemisia ketone (97 % pure) from Sigma-Aldrich. The proportions of the identified compounds were calculated using a calibration range from 10 to 1250 mg/L) in MeOH except for camphor (10 to 2500 mg/L).

3. RESULTS

The essential oils obtained from the various samples of *A. annua* in this study are: alpha pinene, camphene, limonene, alpha terpinene, beta pinene, Carene, eucalyptol, artemisia ketone, copaene, caryophyllene, camphor, menthol and alpha terpineol. Their structures are shown on table 1 and their percentage composition on table 2. Camphor was found to be the major constituent in all the samples analysed with its concentration ranging between 35.67 and 81.50%.

Table 1: Structures of volatiles from a sample of *A. annua* analyzed by GC-MS

Essential oil [retention time (min)]	**Essential oil [retention time (min)]**
 Alpha pinène (**6**) : (8,81)	 Camphene (**7**) : (10,02)
 Limonène (**8**) : (13,40)	 Alpha terpinène (**9**) : (12,82)
 Beta pinène (**10**) : (11,12)	 Carène: (**11**) : (12,14)
 Eucalyptol (**3**) : (13,68)	 Artemisia cétone (**12**) : (16.73)

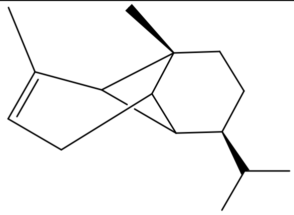
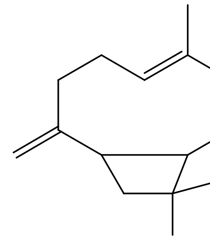
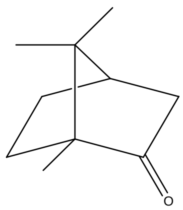
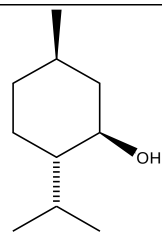
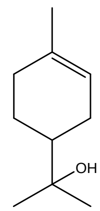
 Copaene (**13**) : (19,97)		
		Caryophyllene (**14**) : (22,08)
 Camphre (**5**) : (20,72)	 Menthol (**4**) : (22,36)	 Alpha terpinéol (**15**) : (23,34)

Table 2. Percentage (%) composition of the essential oils of *A. annua*from various localities in Cameroon and from Luxembourg

		Composition (%) of essential oil							
RI[a]	Compound	Bang[b]	Bang-Fok	Band	Bafo	Mbou	Dsch	Bame	Luxe
8.81	α-Pinene	2.9	1.22	0.5	2.07	1.48	2.45	0.57	0.85
10.02	Camphene	1.09	3	1.91	3.93	2.52	2.16	2.53	2.25
13.4	Limonene	0.13	0.72	0.09	0.06	0.13	0.06	0.16	1.28
12.82	α-Terpinene	0.17	0.14	0.14	0.15	0.1	0.13	0.23	0.25
11.12	β-Pinene	2.9	1.22	0.5	2.07	1.48	2.45	0.57	0.85
12.14	3-Carene	0.05	0.01	0.16	0.03	0.01	0.01	0.44	0.02
13.68	Eucalyptol	2	4.9	5.54	5.58	3.88	2.04	0.16	10.44
16.73	Artemisia ketone	0'05	0	0	0.06	0	0.01	0.01	8.5
19.97	Copaene	1.9	3.4	3.8	1.84	3.58	3.72	4.61	14.27
20.72	Camphor	81.5	62.6	65.67	73.94	61.92	63.15	61.66	35.67
22.08	Caryophyllen e	3.55	16.6	15.3	7.05	17.71	15.91	17.87	18.45
22.36	Menthol	0.1	0.04	0.75	0.05	0.23	0.55	0.5	0.16
23.34	α-Terpineol	3.68	6.04	5.4	3.07	6.62	7.3	10.27	7.02

Bangangte (Bang), Bangang-Fokam (Bang-Fok), Bandjoun (Band), Bafoussam (Bafo), Mbouda (Mbou), Dschang (Dsch), Bamenda (Bame), Luxemburg (Luxe). [a] RI in min. [b] Leaves from plants of second generation.

Figures 1 - 13 show the concentrations (mg/kg) of the essential oils of *A. annua* obtained from various localities in Cameroon and from Luxembourg. Artemisia ketone was found to be present in a quantifiable amount only in the sample obtained from Luxembourg. The concentrations of Limonene, eucalyptol and copaene were also found to be significantly higher in the Luxembourg samples when compared with samples from various localities of Cameroon. Camphor concentration was however found to be significantly higher in the Cameroon samples compared to the Luxembourg samples as mentioned earlier. The concentration of menthol was significantly higher in the Bamenda, Dschang and Bandjoun villages of Cameroon when compared to other

Localities. Furthermore Carene concentration was highest in Bamenda, Limolene in Luxembourg and Bangang-Fokam, Alpha terpineol in Bamenda and Beta pipene in Dschang (Foto) and Bandjoun.

Figure 1: Concentration (mg/kg of leaf) of Alpha Pinene in *Artemisia annua* leaves obtained from various Localities

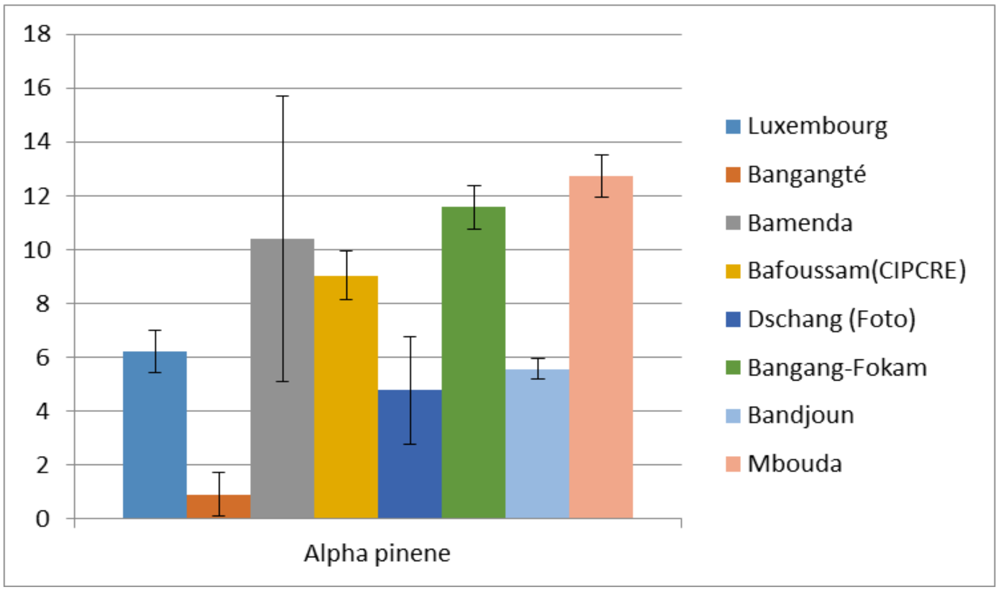


Figure 2: Concentration (mg/kg of leaf) of Camphene in *Artemisia annua* leaves obtained from various Localities

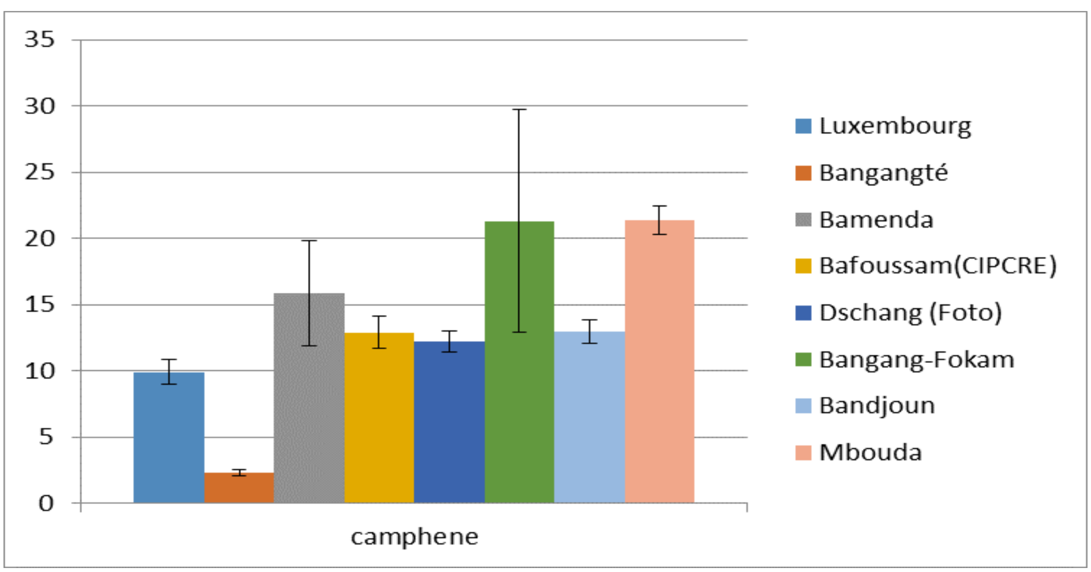


Figure 3: Concentration (mg/kg of leaf) of Carene in *Artemisia annua* leaves obtained from various Localities

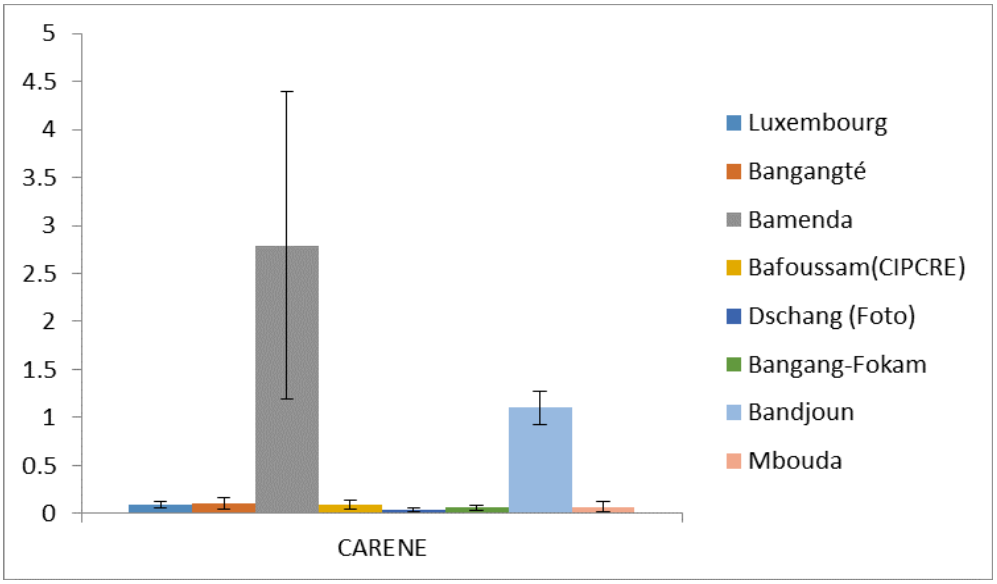


Figure 4: Concentration (mg/kg of leaf) of Alpha Terpinene in *Artemisia annua* leaves obtained from various Localities

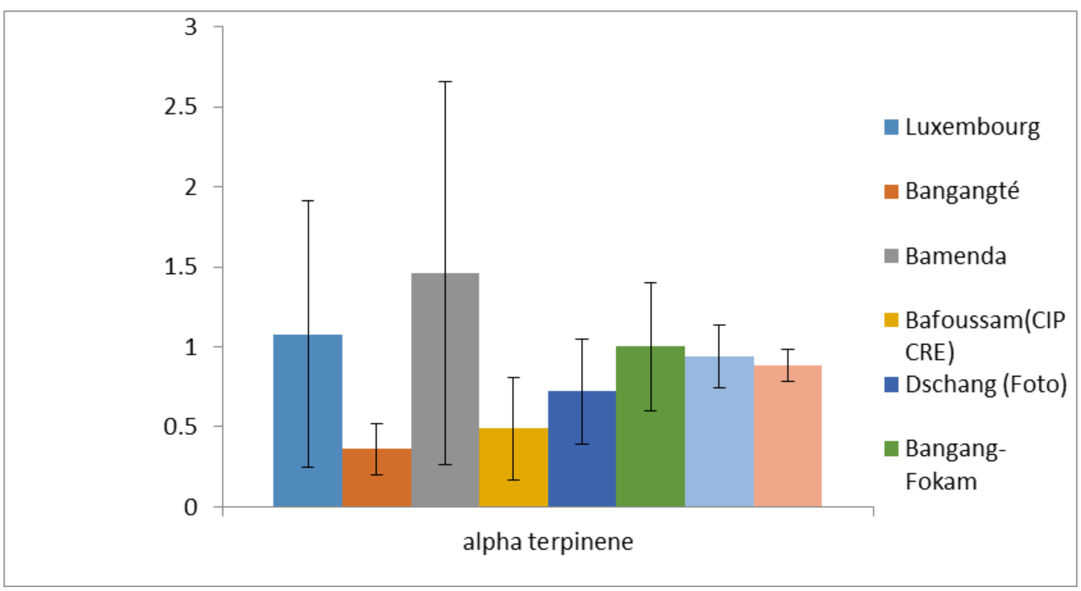


Figure 5: Concentration (mg/kg of leaf) of Limonene in *Artemisia annua* leaves obtained from various Localities

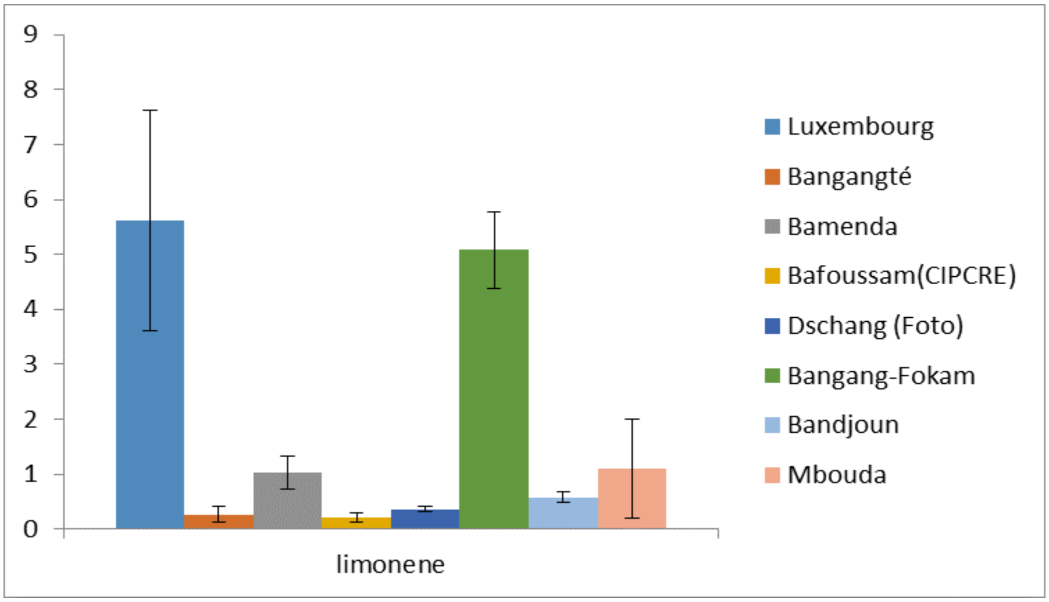


Figure 6: Concentration (mg/kg of leaf) of Eucalyptol in *Artemisia annua* leaves obtained from various Localities

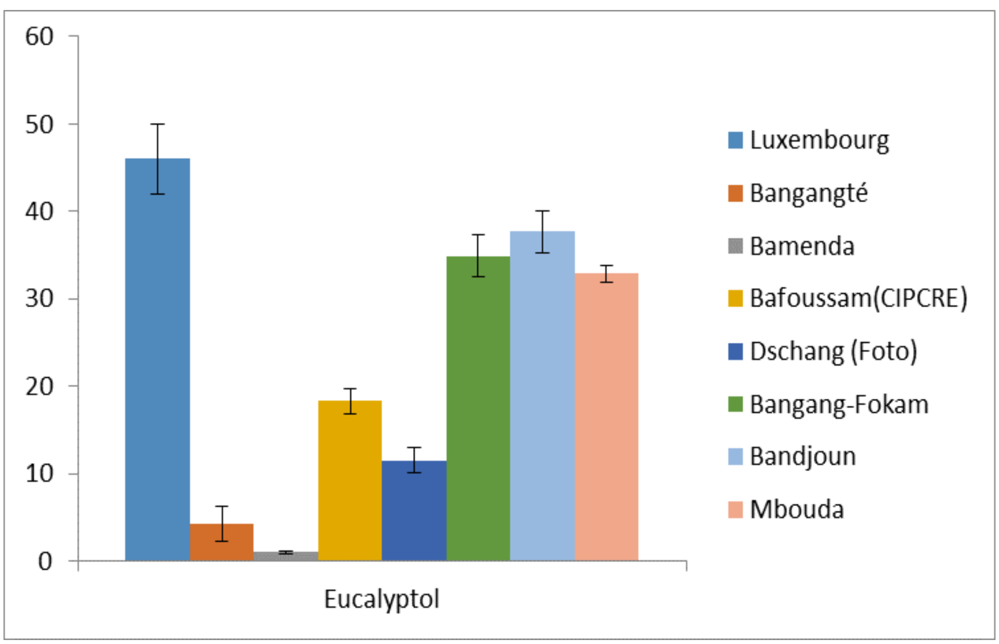


Figure 7: Concentration (mg/kg of leaf) of Artemisia Ketone in *Artemisia annua* leaves obtained from various Localities

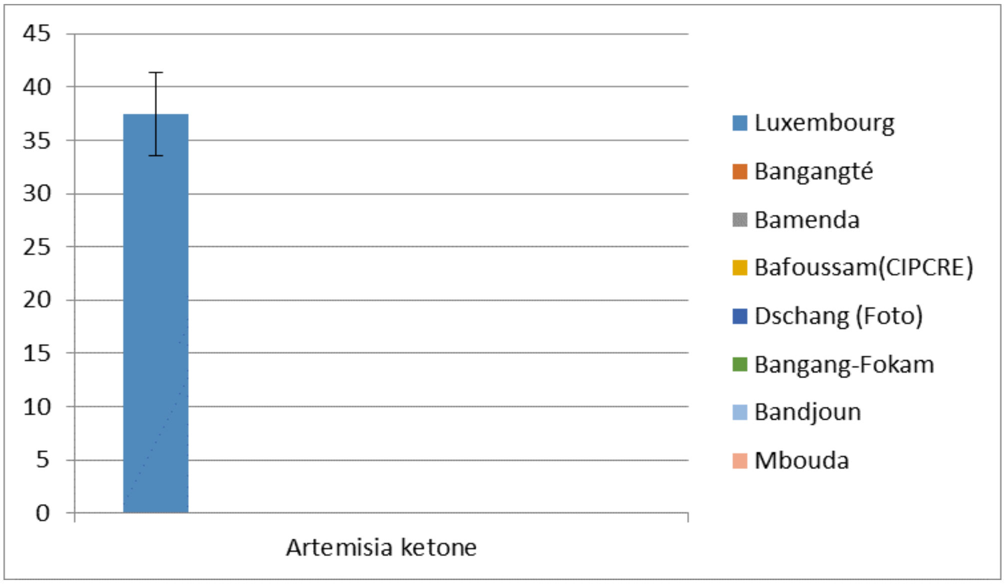


Figure 8: Concentration (mg/kg of leaf) of Copaene in *Artemisia annua* leaves obtained from various Localities

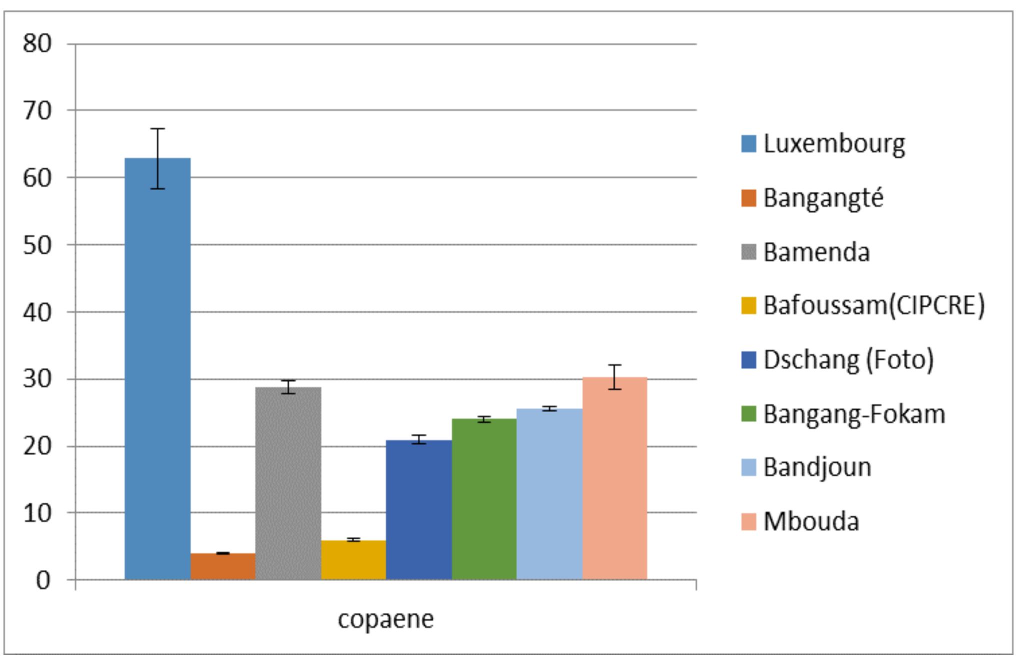


Figure 9: Concentration (mg/kg of leaf) of Camphor in *Artemisia annua* leaves obtained from various Localities

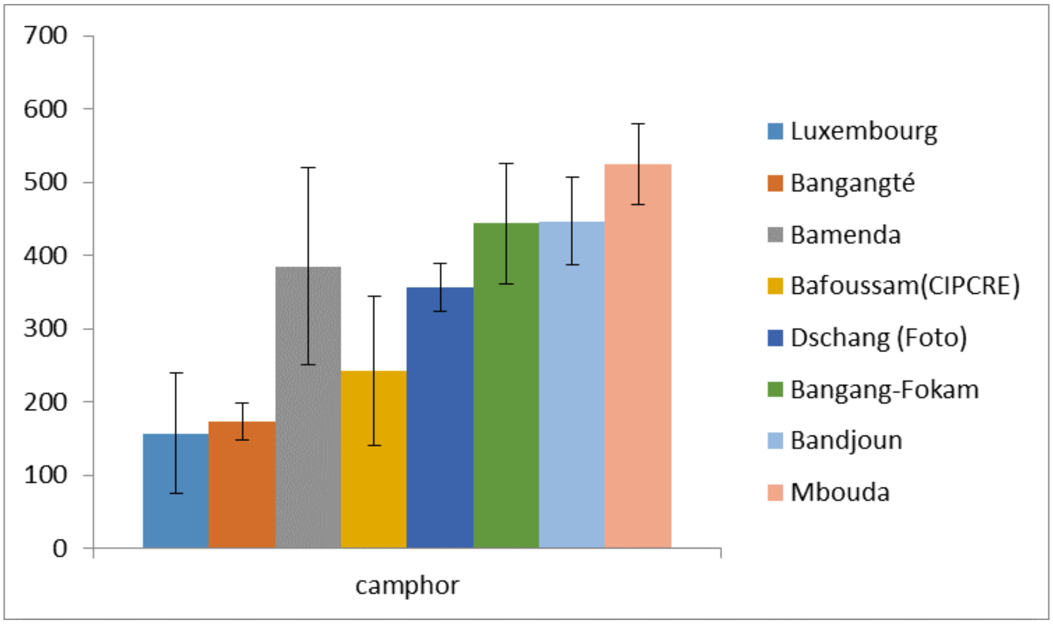


Figure 10: Concentration (mg/kg of leaf) of Caryophylene in *Artemisia annua* leaves obtained from various Localities

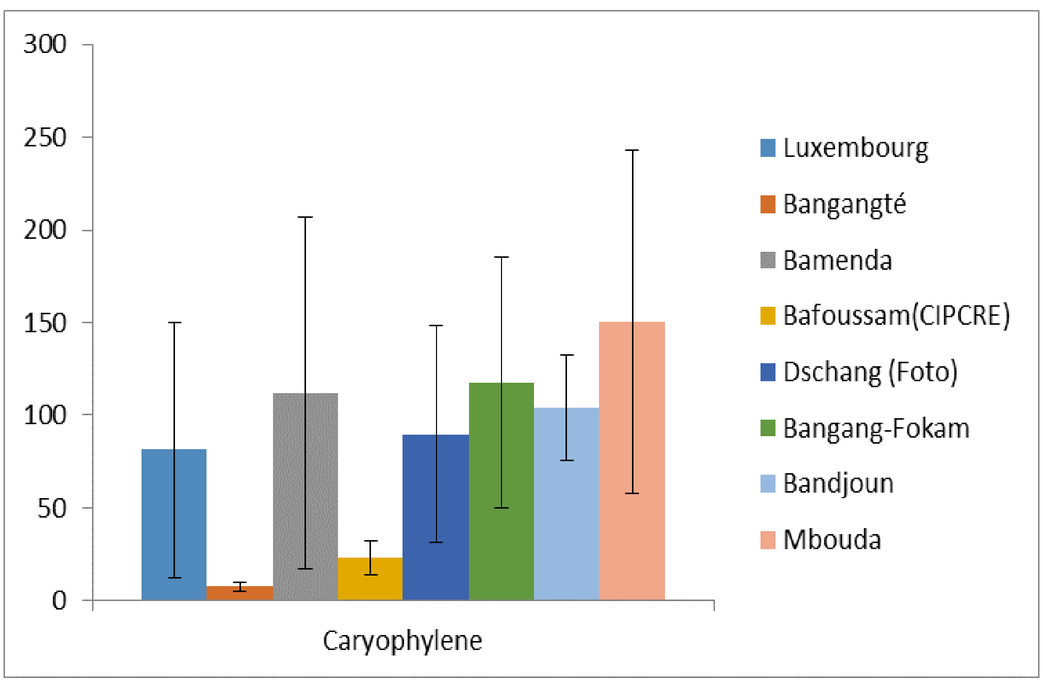


Figure 11: Concentration (mg/kg of leaf) of Menthol in *Artemisia annua* leavesobtained from various Localities

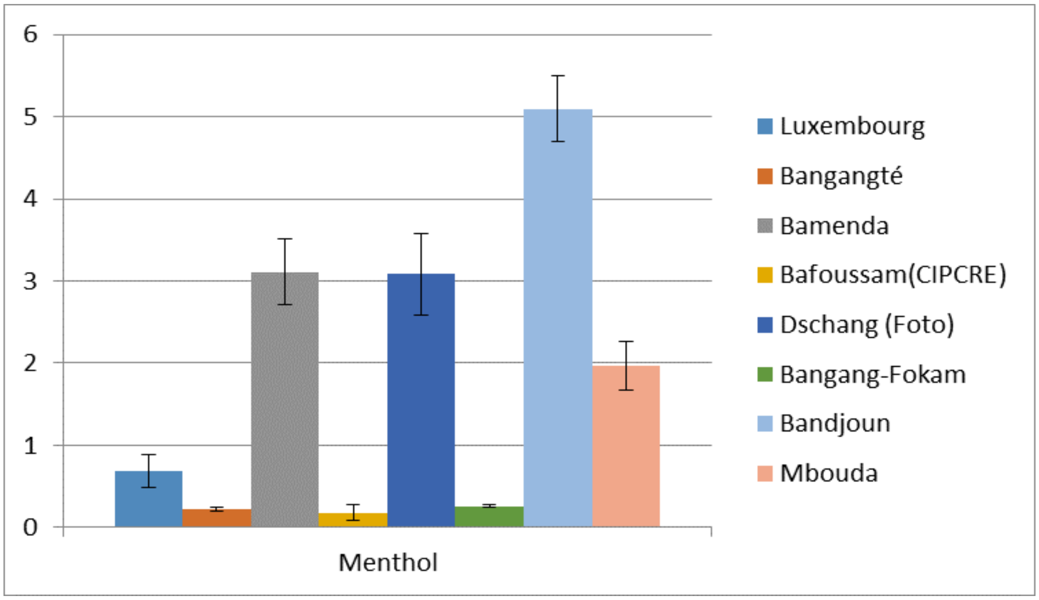


Figure 12: Concentration (mg/kg of leaf) of Alpha Terpineol in *Artemisia annua* leaves obtained from various Localities

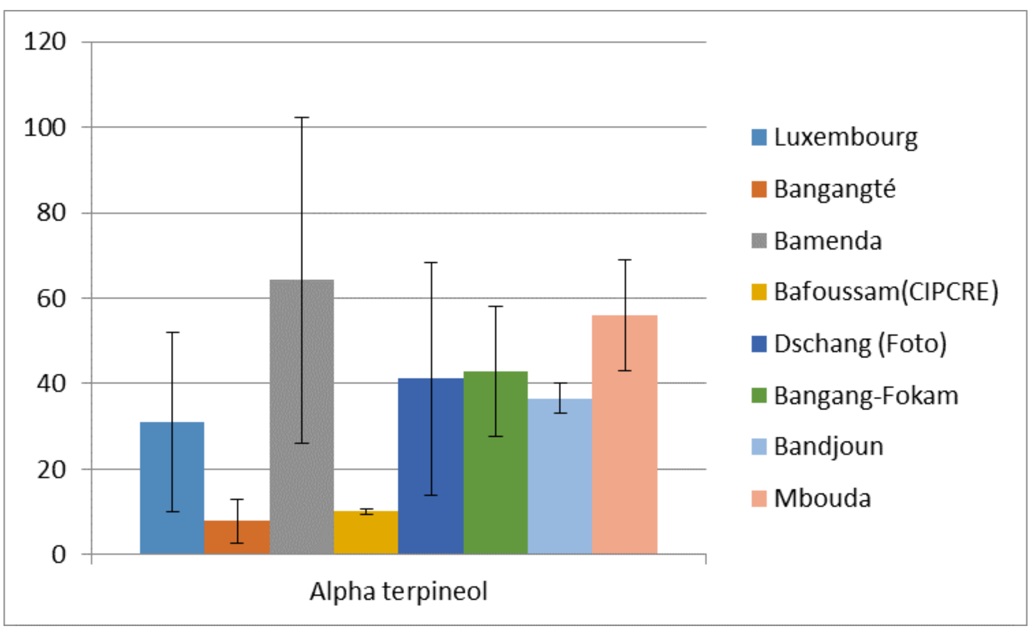
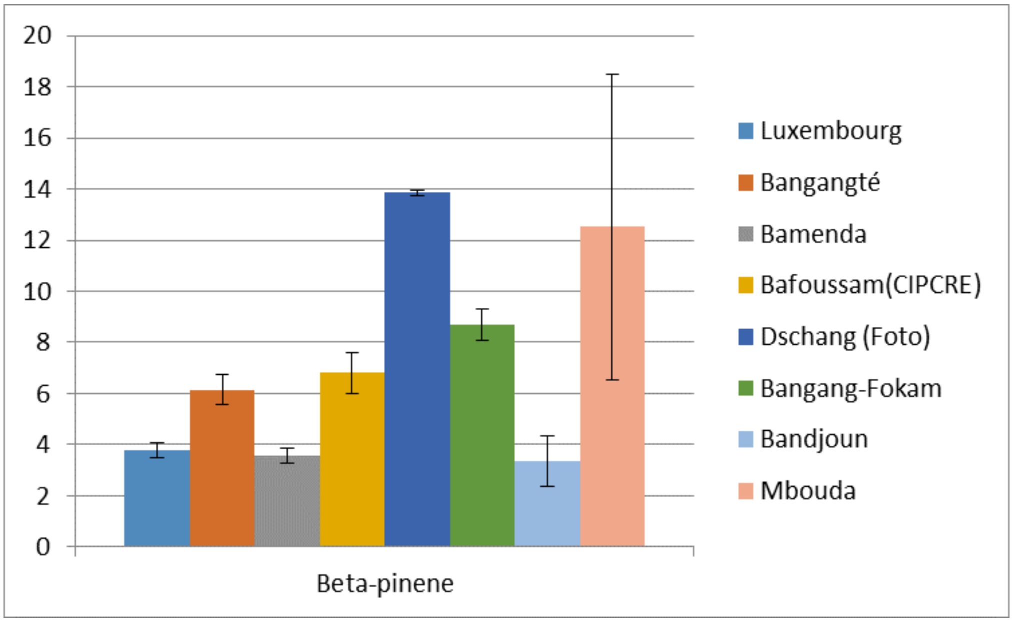


Figure 13: Concentration (mg/kg of leaf) of Beta-pipene in *Artemisia annua* leaves obtained from various Localities



4. DISCUSSION

The chemical composition of essential oil of *Artemisia annua* as revealed in this study is similar to the yield obtained from *Artemisia annua* from Iran (Verdian-Rizi*et al., 2008*) and Bulgaria (Tzenkova*et al., 2010*). Camphor was also found to be the major constituent (>50%) in the plant samples from these countries. With the exception of Luxembourg sample with a concentration of 35.67%, the concentrations of camphor in all the samples analysed in this study were above 60%. *A. annua* from Cameroon villages especially Bagante (with 81.50% yield)could therefore be a rich source from where Camphor could be extracted. Our results compared with those from other countries showed that *A. annua* adapted well in Cameroon.

The essential oils from *Artemisia annua* play a major role in the numerous therapeutic and environmental benefits of the plant and also enhance the bioavailability of its antimalarial component artemisinin. α-Pipene is a terpene with antimalarial activity similar to that of quinine (Seatholo, 2007), (van Zyl*et al.*, 2006). In plants, α-pinenes and its isomer β- pinenes show fungicidal activity and have been used for centuries to produce flavors and fragrances. Several biological activities are associated with pinenes, including use as a natural insecticide (da Silva*et al., 2012*).Camphene, a bicyclic monoterpene used in the manufacture of synthetic camphor has been reported to reduce plasma cholesterol and triglycerides in hyperlipidemic rats independently of HMG-CoA reductase activity (Galle *et al.*, 2016). α-terpinene is used as a fragrance and it possesses antioxidant activity. Limonene, part of the so-called cineole cassette (Raguso *et al.*, 2006), is suggested to arrests isoprenoid biosynthesis in Plasmodium (Goulart *et al.*, 2004)and development at the ring and trophozoite stages (Moura *et al.*, 2001)of the parasite. It is also reported to inhibit protein isoprenylation in *P. falciparum*, arresting parasite development within 48 h of treatment (Moura *et al.,* 2001)and its metabolites remain in the plasma for at least 48 h (Miller*et al.*,2001)making it an important agent for the elimination of gametes and malaria transmission.

Artemisia ketone, a major constituent of A. annua, is suggested to play a role in hemozoin formation. It prevents plasmodium from converting toxic heme to β- hematin and hemozoin, which is non-toxic to the parasite thereby inhibiting cell-mediated immunity against the parasite (Akhtar*et al.*, 2013). Carene is a bicyclic monoterpene with a sweet and pungent odour. It is a raw material for perfumes, cosmetics and flavours. Camphor, a terpenoid with a strong aromatic odour, is used as a stimulant, antispasmodic, antiseptic, decongestant, anesthetic, sedative and nervous pacifier, antineuralgic, anti-inflammatory, disinfectant and insecticide. Copaene is a tricyclic sequisterpene which is of economic significance due to its strong attraction to an agricultural pest and caryophyllene is a bicyclic sequisterpene with a spicy woody odor. Sequisterpenes are analgesic, antifungal and antibacterial.

Menthol is a monoterpene which is widely used in cosmetics, as a flavoring agent, an insect repellent and as an intermediate in the production of other compounds. Furthermore menthol has been reported to exhibit cytotoxic effects in cancer cells, induce reduction of malignant cell growth, and engage in synergistic excitation of GABA receptors and sodium ion channels resulting in analgesia [Farco and Grundmann, 2013). Eucalyptol (1,8-Cineole), a cyclic ether and monoterpene is a strong inhibitor of the pro-inflammatory cytokines TNF-a, IL-6, and IL-8 (Juergens*et al.,* 2014). It inhibits the growth and development of chloroquine-resistant and chloroquine-sensitive Plasmodium strains at the early trophozoite stage (Su*et al.*, 2008). Its possible use as an antimalarial inhalant has also been suggested (Kovar*et al.,* 1987). Alpha terpineol is a monoterpenoid alcohol known to enhance the permeability of skin to lipid- soluble compounds (Williams and Barry, 1991). It has been reported to exhibit anti-proliferative effects on human erythroleukaemic cells (Lampronti*et al.*, 2006) and to inhibit the growth of tumour cells through a mechanism that involves inhibition of the NF-κB pathway (Hassan*et al.*, 2010). It has also been described to have anti-inflammatory properties (Held*et al.*, 2007), as it was found to be a potent inhibitor of superoxide production, selectively regulating cell function during inflammation [Brand*et al.*,2001). Alpha terpineol has also been shown to have antibacterial [Kotan*et al.*,2007)and antifungal activities [Pitarokili*et al.*, 2002).

The variations in the chemical composition of *A. annua* from various localities in this study which is in line with previous reports from Iran and Bulgaria has been suggested to be as a result of differences in the geographical location and culture conditions of the plants (Verdian-Rizi*et al.*,2008), (Bhakuni *et al.*, 2001), (Woerdenbag *et al.*, 1993), (Tzenkova, *et al.,* 2010). The variations have been suggested to affect the numerous biological activity of the plant.

Samples from Bangangte was found to give the smallest yield of all constituents studied except camphor and this could be explained by the fact that, the plants collected were the product of the second generation. This indicates that it would be preferable to perform a cultivation of *A. annua* by cuttings rather than by using the seeds from the previous generation.

CONCLUSION:

There were variations in the chemical composition of the essential oils obtained from various localities in Cameroon and from Luxembourg. These variations which could affect the biological activity of the plant are suggested to be as a result of differences in the geographical location and culture conditions of the plants. *Artemisia annua* is well acclimated in Cameroon and could be used for extraction of Camphor.

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