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Research Article

# CHEMICAL COMPOSITION, ANTIRADICAL AND ANTI-INFLAMMATORY ACTIVITIES OF FOUR ANNONACEAE FROM BENIN

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**ABSTRACT**
The chemical composition of the essential oils obtained by hydrodistillation from fresh leaves of four Annonaceae: *Annona muricata, Annona squamosa, Monodora myristica and Xylopia aethiopica* growing wild in Benin were analyzed by GC and GC/MS. Twenty four compounds were identified and quantified in the essential oil of *Annona muricata* with isocaryophyllene (20.2%); β-caryophyllene (16.1%); δ–cadinene (11.4%), β–elemene (8.9%); α–muurolene (6.9%) as major components. Twenty nine compounds were identified and quantified in the essential oil of *Annona squamosa* which contained isocaryophyllene (24.9%), camphene (10.2%), β–caryophyllene (2.6%), epi-α–cadinol + epi–α–muurolol (9.2%) as prominent components. Essential oil of *Monodora myristica* screened contained twenty four components identified and quantified with α–phellandrene (65.5%), α–pinene (6.2%) as main compounds. Thirty three compounds were identified and quantified in the essential oils of *Xylopia aethiopica,* the major compounds being p-cymène (16.0%), sabinene (12.6%), terpinen-4-ol (11.3%), β–elemene (10.6%) and β–pinene (7.1%). The antiradical and anti-inflammatory activities of these oils were found to be low.

**Keywords**: Antiradical activity, anti-inflammatory activity, essential oils, annonaceae.

## INTRODUCTION

*Annona muricata* is a specie of tropical America and the Antilles, nowadays acclimated in several tropical areas, particularly in Africa (Benin, Togo and Congo), in the Comoros and Domenica for its edible fruit (corossolier). Some tribes of low Casamance use plasters of the leaves crushed for the wounds of the circumcision [1]. It is used in Benin in many therapeutic preparations for the treatment of hypertension, hypotension, cardiac affections, nervous crises, giddinesses, epilepsy, icterus, convulsions, fever, cough, diarrhoeas and chronic dysentery [1-4]. It is also used to fight against insomnia and for treatment of kidney calcul. Moreover, *Annona muricata* is used in the treatment of cramps and the heartburn etc.[5]. The seed extracts of this species presented an insecticidal activity [1]. Ethanol extract of *A. muricata* leaves can be an active source of substances with antinociceptive and anti-inflammatory activities [6].

*Annona squamosa* was used against the giddinesses after trituration and inhalation of the fresh leaves [2]. In Benin, it was used in case of insomnia, like insecticide [4].

*Monodora myristica* was used in the treatment of measles, as condiment in many medicinal recipes, like vermifuge by the population of Gabon, Congo and Togo [7, 9]. In Benin, the seeds were also been used in the treatment of cough. They were employed in case of convulsions [4].

Traditionally in Benin, *Xylopia aethiopica* was used as aromatic condiment "pepper of Ethiopia" and like cough reliever [4]. It is used for the treatment of bronchitides, rheumatisms, dysentery, but also to stimulate the fertility among women [1].

Many studies were carried out on this four species in the phytochemical, pharmacological and agroalimentary ways. The main chemical studies were linked to the sugars, the lipids, the protids, the tannins, the sterols and alkaloids isolated from fruits, seeds, leaves and barks of *Annona muricata, Annona squamosa, Monodora myristica* and *Xylopia aethiopica*. For *Annona muricata,* five compounds volatile were identified in the extract obtained from the fruits of Mexican origin, namely to it (E)-2-hexenal, methyl caprylate, methyl caproate, acetaldehyde and methyl hexanoate [10]. Some investigations on the volatile part of the fruits of three species of *Annona* of Malaysian origin were carried out. It comes out from this study that the volatile part of the fruit *of Annona muricata* was composed in the majority of esters: methyl (E)-goal-2-énoate (19.7%), methyl (E)-hex-2-énoate (18.4%), and methyl 2-hydroxy hexanoate (5.2%) as well as alcohols, including it (Z)-hex-3-in-1-ol (9.7%) and linalol (9.3%) [11,12]. The decoction of the leaves of *Annona muricata* showed an action against *Escherichia coli* [13]. The constituents were □-caryophyllene (13.6%), □-cadinene (9.1%), epi-□-cadinol (8.4%), □-cadinol (8.3%) [14]. Tkahashi *et al.* [15] showed that the extract of *Annona muricata* of Brazil presented an antibacterial activity.

The analysis results of *Annona squamosa* L. showed that essential oil of the plant studied mainly made of hydrogenated sesquiterpenes were, dominated by isocaryophyllene (13.1%; 24.9%) and □-caryophyllene (2.6%; 13.4%) respectively. The essential oil of *Monodora myristica* (Gaertn.) Dunal. was exclusively terpenic, dominated by □-phellandrene (65.5%), accompanied by p-cymene (4.5%), limonene (4.2%) but also composed of pinenes (□ and □, respectively 6.2 and 4.8%) [16].

The oil of *Xylopia aethiopica* (Dunal) A. Rich was dominated by hydrocarbonated terpenes (69.2%) with monoterpenes in majority, in particular the sabinene (12.6%), p-cymene (16.0%), and limonene (7.9%). Among the oxygenated derivatives the terpinen-4-ol was most abundant (11.3%) [17]. In the essential oil of the leaves, terpinen-4-ol (30.8%), sabinene (14.7%), myrtenol (9.1%), □-terpinene (6.2%), 1-8 cineole (5.3%), □-pinene (4.7%) [18-24] were the major compounds.□□-pinene (23.6%), □-pinene (11%), sabinene (9.8%), germacrene D (8.3%) and 1,8 cineole (8.2%) were the major constituents of the extract from the fruits collected in Togo [25].The essential oil of *Xylopia aethiopica* presents the biological activities (antimicrobial, insecticidal, larvicidal, ovicidal, antifungal, antioxydant, cytotoxic and anti-inflammatory) [18-30]. Although these plants are largely studied, to our knowledge, no study was conducted on the antiradical and anti-inflammatory of essential oils extracted from these plants in Benin. These activities of the following extracts must be verified in order to measure their potential for their valorization.

The aims of the present work were to evaluate the antiradical, anti-inflammatory effect of some essential oils of the four annonaceae, *Annona muricata, Annona squamosa*, *Monodora myristica* and *Xylopia aethiopica* of different areas and their chemical composition.

## MATERIALS AND METHODS

### Plants material and isolation of the essential oils

The plant material was collected in three areas of Benin at Sikecodji (*Annona squamosa*) February 2005, at Gbegamey (*Annona muricata*) in February 2005 and at Abomey-Calavi (*Monodora myristica*, *Xylopia aethiopica*), in May 2003, July 2003. A voucher specimen of each plant was deposited in the Herbarium of the University of Abomey-Calavi. Batches of 200 g of fresh leaves were submitted to hydrodistillation for 2h using a Clevenger-type apparatus; after decantation, the oils were dried over anhydrous $Na_2SO_4$ and stored in sealed vials below 10°C until using.

**Chemical analyses of essential oils**

Quantitative and qualitative analyses of the essential oils were carried out by gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS).

GC/FID analyses were performed using a Varian CP-3380 GC equipped with a DB1 (100% dimethylpolysiloxane) fitted with a fused silica capillary column (30 m x 0.25 mm, film thickness 0.25 µm) and Supelcowax 10 (polyethylene glycol) fused capillary column (30 m x 0.25 mm, film thickness 0.25 µm); temperature program 50°-200°C at 5°C/min, injector temperature 220°C, detector temperature 250°C, carrier gas $N_2$ at a flow rate of 0.5 $mL.min^{-1}$. Diluted samples (10/100, v/v, in methylene chloride) of 2.0 µL were injected manually in a split mode (1/100). The percentage compositions were obtained from electronic integration measurements without taking into account relative response factors. The linear retention indices of the components were determined relatively to the retention times of a series of *n*-alkanes ($C_9$-$C_{20}$).

GC/MS analyses were performed using a Hewlett Packard apparatus equipped with a HP1 fused silica column (30 m x 0.25 mm, film thickness 0.25 µm) and interfaced with a quadruple detector (Model 5970). Column temperature was programmed from 70° to 200°C at 10°C/min; injector temperature was 220°C. Helium was used as carrier gas at a flow rat of 0.6 $mL.min^{-1}$, the mass spectrometer was operated at 70 eV. 2.0 µL of diluted samples (10/100, v/v, in methylene chloride) were injected manually in the split mode (1/100).

The identification of individual compounds was based on the comparison of their relative retention times with those of authentic samples on the DB1 column and by matching the linear retention indices and mass spectra of peaks with those obtained from authentic samples and/or the NBS75K.L and NIST98.L libraries and published data [31, 32].

**Biological evaluation: *Free radical-scavenging and anti-inflammatory activities***

The antiradical scavenging activity of the oil samples was tested using 2,2-diphenyl-1-1-picrylhydrazyl (DPPH) following the Mellors and Tappel method [33], adapted to essential oils screening [34]. Their potential anti-inflammatory activities were evaluated by testing their inhibitory effect on soybean lipoxygenase activity comparatively to that of nordihydroguaiaretic acid following the procedure previously described [35].

**Statistical analysis**

Data were subjected to analysis of variance (ANOVA). They were expressed as the mean ± standard error of triplicate measurements; standard deviations did not exceed 5 %.

## RESULTS AND DISCUSSION

**Chemical composition of the essential oils**

The yields of essential oils obtained by hydrodistillation of fresh leaves of four annonaceae collected in three locations of Benin are given in Table 1. Weak yields lower than 0.1% for *Annona muricata* and *Xylopia aethiopica* were noted. Also, weak yields were obtained for *Annona squamosa* (0.2%) and *Monodora myristica* (0.5%).

The chemical compositions of these essential oils are presented in Table 2. Globally, the essential oils were dominated by hydrogenated monoterpens and sesquiterpens hydrocarbons.

The sample of *Annona muricata* was dominated by hydrogenated sesquiterpens, in particular by the isocaryophyllene (20.2%), the □-caryophyllene (16.1%) and the □-cadinene (11.4%). We thus found a chemical composition very comparable with those obtained for the essential oils of the leaves extracted from the species collected in Côte d'Ivoire [36], in Benin [37], in Cameroun [38] or in Gabon [39] and different from that obtained [14] by Kossouho *et al*. 2007. The analysis of the results from *Annona squamosa* L. showed that the sample of *Annona squamosa* studied mainly composed of hydrogenated sesquiterpens were, dominated by the isocaryophyllene (24.9%) and the □-caryophyllene (13.4%). The essential oil *Monodora myristica* (Gaertn.) Dunal. was exclusively terpenic, dominated by □-phellandrene (65.5%), accompanied by p-cymene (4.5%), limonene (4.2%) but also of pinenes (□ and □, respectively 6.2 and 4.8%). We thus found a chemical composition quite similar to those described previously. The essential oil of *Xylopia aethiopica* (Dunal) A. Rich is dominated by hydrogenated terpenes (69.2%) with monoterpenes in majority, in particular the sabinene (12.6%), p-cymene (16.0%), and limonene (7.9%). Among the oxygenated derivatives the terpinen-4-ol was most abundant (11.3%). We found an essential oil similar to the essential oil of the fruits generally described, in particular that of Cameroun. However, the sample of the leaves collected in Benin and previously studied [17] was characterized by a high rate much higher in □-pinene. The most abundant sesquiterpene was the □-elemene, that to correlate the chemical structure with the elemol, mainly described previously in Benin sample.

**Table 1: Yields (w/w percentage) of essential oils obtained from fresh leaves of essential oils obtained from fresh leaves of four Annonaceae from Benin**

| Samples | Date and place of harvest | Yields (% w/w) |
|---|---|---|
| *Annona muricata* | February 2005 (Gbegamey) | 0.06 |
| *Annona squamosa* | February 2005 (Sikecodji) | 0.20 |
| *Monodora myristica* | May 2003 (Abomey-Calavi) | 0.50 |
| *Xylopia aethiopica* | July 2003 (Abomey-Calavi) | 0,07 |

**Table 2: Chemical composition of essential oils of leaves of four Annonaceae from Benin**

| RI* | Components | *A.m.* | *A.s.* | *M.m.* | *X.a.* |
|---|---|---|---|---|---|
| | | % | | | |
| 927 | □-thujene | - | - | 2.4 | 1.9 |
| 936 | □-pinene | - | 3.8 | 6.2 | 3.5 |
| 951 | camphene | - | 10.2 | - | - |
| 977 | sabinene | - | - | 0.1 | 12.6 |
| 979 | myrcene | - | 0.3 | - | - |
| 985 | □-pinene | - | - | 4.8 | 7.1 |
| 1009 | □-phellandrene | - | - | 65.5 | - |
| 1016 | □-terpinene | - | - | 0.1 | - |
| 1020 | p-cymene | - | - | 4.5 | 16.0 |
| 1029 | limonene | - | 2.0 | 4.2 | 7.9 |
| 1040 | (E)-□-ocimene | - | - | 0.1 | - |
| 1056 | □-terpinene | - | - | 0.1 | - |
| 1064 | *trans*-oxyde de linalol (furanoid) | - | - | - | 0.2 |
| 1087 | *cis*-oxyde de linalol (furanoid) | - | - | - | 0.1 |
| 1089 | linalol | - | 0.2 | 1.6 | 2.4 |
| 1119 | *cis*-p-menth-2-èn-1-ol | - | - | - | 0.6 |
| 1136 | *trans*-p-menth-2-èn-1-ol | - | - | - | 0.4 |
| 1140 | *trans*-pinocarveol | - | - | - | 0.7 |
| 1177 | methyl chavicol | 0.4 | - | - | - |
| 1179 | terpinen-4-ol | - | - | - | 11.3 |
| 1186 | □-terpineol | - | - | 0.1 | 2.6 |
| 1197 | sabinol | - | - | 0.4 | - |
| 1282 | acetate de bornyle | - | 0.2 | - | - |
| 1331 | bicycloelemene | - | 0.1 | - | - |
| 1334 | □-elemene | 4.5 | 1.9 | - | 2.8 |
| 1358 | □□cubebene | - | - | 0.4 | - |
| 1379 | □-copaene | 1.0 | 0.2 | 0.1 | 0.6 |
| 1390 | □-elemene | 8.9 | 4.7 | - | 10.6 |
| 1412 | isocaryophyllene | 20.2 | 24.9 | - | - |
| 1428 | □-caryophyllene | 16.1 | 2.6 | 0.1 | - |
| 1444 | □□copaene | - | 0.2 | - | - |
| 1457 | aromadendrene | - | 0.1 | - | - |
| 1460 | □-humulene | 3.4 | 3.5 | - | - |
| 1468 | alloaromadendrene | 0.8 | - | - | - |
| 1476 | □-elemene | - | - | - | 2.3 |
| 1476 | □-muurolene | 1.8 | - | 0.1 | 1.3 |

| | | | | | |
|---|---|---|---|---|---|
| 1479 | □□curcumene | - | 2.5 | - | - |
| 1481 | germacrene D | 1.1 | 4.1 | - | 1.0 |
| 1491 | □-selinene | 1.1 | 6.6 | - | 0.8 |
| 1500 | □□muurolene | 6.9 | - | 0.8 | - |
| 1512 | bicyclogermacrene | - | 2.5 | - | - |
| 1514 | □-cadinene | 1.1 | 2.4 | 2.1 | - |
| 1526 | □-sélinene | - | - | - | 0.5 |
| 1536 | □□cadinene | 11.4 | 3.2 | 3.6 | 0.2 |
| 1550 | □-cadinene | - | - | 0.1 | - |
| 1550 | calamenene | - | - | - | 0.1 |
| 1544 | elemol | 0.7 | - | - | 2.9 |
| 1552 | muurol-5-en-4-ol | 1.3 | - | - | - |
| 1558 | acide laurique | - | 1.1 | - | - |
| 1560 | spathulenol | 0.2 | 2.7 | - | - |
| 1566 | salvialenone | - | - | - | 0.6 |
| 1590 | germacrene D-4-ol | - | - | 0.2 | - |
| 1592 | oxyde de cayophyllene | 4.1 | 2.2 | - | 1.5 |
| 1616 | oxyde d'humulene II | 0.8 | - | - | 1.1 |
| 1628 | 1-epicubenol | 1.0 | - | - | - |
| 1639 | isospathulenol | - | 2.1 | - | 1.1 |
| 1642 | □-eudesmol | - | - | - | 0.2 |
| 1643 | epi□□□muurolol + epi-□□cadinol | 5.3 | 9.2 | 0.2 | 0.3 |
| 1661 | □□cadinol | 4.6 | 3.8 | 0.1 | 0.4 |
| 1669 | □-eudesmol | - | - | - | 1.3 |
| 1700 | heptadecane | 0.4 | | - | - |
| 1778 | geraniate de methyle | 0.4 | - | - | - |
| Monoterpens hydrogenated | | - | 16.3 | 88.0 | 49.0 |
| Oxygenated monoterpens | | 0.4 | 0.4 | 2.1 | 18.3 |
| Sesquiterpens hydrogenated | | 78.3 | 59.5 | 7.3 | 20.2 |
| Oxygenated sesquiterpens | | 13.6 | 20.0 | 0.5 | 9.4 |
| Aromatic compounds | | 0.4 | - | - | - |
| Aliphatic derivatives | | 0.4 | 1.1 | - | - |
| Total | | 93.1 | 97.3 | 97.9 | 96.9 |

*A.m.* = *Annona muricata* L.; *A.s.* = *Annona squamosa* L.; *M. m.* =*Monodora myristica* ; *X.a.* = *Xylopia aethiopica;*
RI*, Retention index relative to n-alkanes ($C_9$-$C_{20}$) on a DB1 capillary column (100% dimethylpolysiloxane);

## Identification methods

GC, identification based on retention times of authentic compounds
MS, identification based on computer matching of the mass spectra of peaks with NBS75 K.L, NIST98.L libraries and published data [18, 19].
RI, tentative identification based on comparison of retention index of the compounds with published data [31, 32].

## Antiradical activity

Free radical scavenging activities were observed for the six oils samples; they were compared to those of the commercial antioxidant BHT (butylated hydroxytoluene), which was widely used as a reference.

**Table 3: Screening of the samples of four essential oils of leaves of four annonaceae at 5g/L**

| Essential oil samples | Percentage of inhibition |
|---|---|
| *Annona squamosa* | 66.1 ± 3.3 |
| *Annona muricata* | 10.1 ± 0.5 |
| *Monodora myristica* | 19.3 ± 0.9 |
| *Xylopia aethiopica* | 12.1 ± 0.6 |

**Table 4: Antiradical activity of essential oils of leaves of four annonaceae from Benin**

| Essential oil samples | $SC_{50}$ (g/L) |
|---|---|
| *Annona Squamosa* | 5.0 ± 0.2 |
| *Annona muricata* | 10.1 ± 0.5 |
| *Monodora myristica* | 15.0 ± 0.8 |
| *Xylopia aethiopica* | 15.0 ± 0.8 |

For *Xylopia aethiopica*, *Monodora myristica* and *Annona muricata*, the increase in the essential oil concentration didn't affect significally the percentage of trapping of the DPPH we can thus regard them as "slightly active" (Table 3). They were in all the cases of compositions rich in mono and/or hydrocarbonated sesquiterpens that explained this weak activity easily (Figure 1). We obtained at the time of the screening a percentage of 66% for a concentration of 5 g/L for sample 1 of essential oil of *Annona squamosa* collected at Sikecodji. The $SC_{50}$ neighbouring to 5 g/L, thus corresponds to an activity 500 times weaker than that of the BHT ($SC_{50}$ = 7.5 ± 0.45 mg/L) (Table 4). We found for this essential oil, a chemical composition dominated by the hydrogenated terpenic structures, as for the samples classified previously "slightly active". The activity observed, could be due to the presence of one (or several) constituents minority in essential oil (Figure 2).

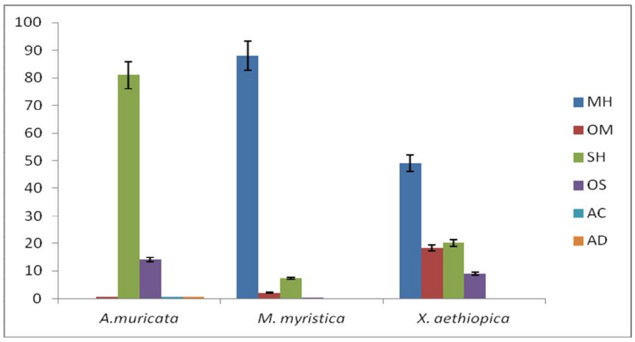


**Fig. 1: Distribution of the terpenoides in essential oils of the leaves of *Annona muricata*, *Monodora myristica* and *Xylopia aethiopica***

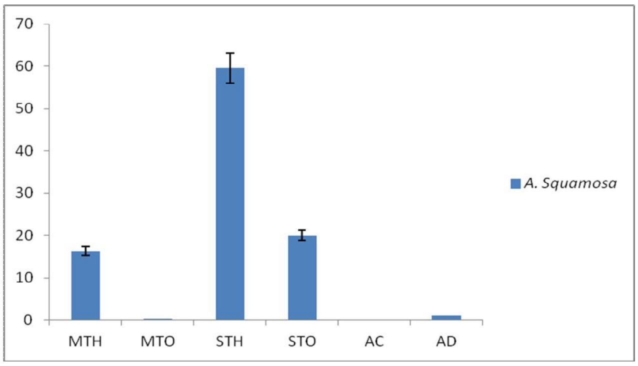


**Fig. 2: Distribution of the terpenoides in essential oils of the leaves of *Annona squamosa***

## Anti-inflammatory activity

The results obtained from the lipoxygenase tests performed on these essential oils were given in table 5.

**Table 5: In vitro inhibition of soybean lipoxygenase by four annonaceae essential oil**

| Essential oil samples | Concentrations (ppm) | Inhibition percentage (%) | $IC_{50}$ (ppm) |
|---|---|---|---|
| *Annona Squamosa* | 10 | 0 ±0.0 | - |
| *Annona muricata* | 100 | 0 ± 0.0 | - |
| *Monodora myristica* | 10 | 28 ± 1.7 | - |
| *Xylopia aethiopica* | 50 | 12 ± 0.7 | - |
| NDGA | 0.75 | 91 ± 5.5 | 0.23 ± 0.01 |

The essential oil samples of *Annona muricata* and *Annona squamosa* did not present any activity of anti-inflammatory drug. The maximum concentration tested is conditioned by the solubility of the sample and its absorptance in the enzymatic solution.
The essential oil of *Annona muricata* was tested with 100 ppm and did not present any inhibiting effect at this concentration. *Annona squamosa* did not present any anti-inflammatory drug activity at 10 ppm, the limiting concentration of its solubility. To 50 ppm we obtained 12% of inhibition (maximum concentration likely to be tested taking into account the solubility of the sample in the medium) for the essential oil of *Xylopia aethiopica*. We found, as in the sample a significant rate of terpinen-4-ol (11.3%) but nothing allowed us to say that the activity observed (average activity) comes from the presence of this compound. Complementary measures would be necessary to confirm this assumption. The sample of *Monodora myristica* could not be evaluated at a concentration > 10 ppm. This sample very rich in hydrogenated monoterpens was far always of this polar. To 10 ppm we obtained 28 % of inhibition (maximum concentration likely to be tested taking into account the solubility of the sample in the medium) for the oil of *Monodora myristica*. It would be interesting to test this sample with higher concentrations in the presence of solubilizing like the cyclodetrines, to confirm the potential activity (average activity) of this sample by the description of a "effect of concentration". We observed at 100 ppm and 10 ppm the essential oils of *Annona muricata* and *Annona squamosa* did not present any inhibition. On the other hand at 50 ppm and 10 ppm essential oils of *Xylopia aethiopica* and *Monodora myristica* displayed a weak inhibition (12% and 28%) which is 7.6 and 3.3 times weaker than that obtained by the NDGA (91%) with 0.75 ppm.

## CONCLUSION

Essential oils compositions of four annonaceae in three different locations in Benin were investigated. We notice that oxygenated and hydrogenated sesquiterpens were observed for *Annona muricata* and *Annona squamosa*. Whereas monoterpens hydrogenated and oxygenated were observed for *Monodora myristica* and *Xylopia aethiopica*. We observed that oils of *Annona muricata* and *Annona squamosa* did not present any inhibition. On the other hand those of *Xylopia aethiopica* and *Monodora myristica* displayed a weak inhibition.

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