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# Chemical Composition and Antibacterial Activities of the Essential Oils from the Leaves of *Premna angolensis* and *Premna quadrifolia*, Two Lamiaceae from Côte d'Ivoire

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**Abstract:- This study was conducted with the objective of contributing to the valorization of aromatic and medicinal plants of Côte d'Ivoire. The essential oil (EO) of the leaves of two species of *Premna, P. angolensis* and *P. quadrifolia* obtained through steam distillation process, were analized through gas chromatography coupled with mass spectroscopy (GC-MS) and their antimicrobial activities was evaluated by agar diffusion method. The yields of EO extracted from the leaves of *P. angolensis* and *P. quadrifolia* are respectively 0.10 ± 0.02% and 0.40 ± 0.02%.**

**99.43% of the total chemical composition of the EO of *P. angolensis* leaves, are composed mainly of hydrocarbon sesquiterpenes (71.90%) followed by oxygenates (14.16%) and monoterpenes (13.22%). The main compounds found are β-caryophyllene (33.07%) and Humulene (10.78%). The EO of *P. quadrifofia* leaves, 99.59% of the composition was identified. It contains hydrocarbon sesquiterpenes (89.15%), followed by oxygenates compounds (8.61%) and monoterpenes (1.83%). The major compounds are α-bulnesene (23.33%), germacrene D (18.83%) and caryophyllene (18.06%). The antimicrobial tests have shown that EO of *P. quadrifolia* leaves has no activites on studied strains and *P. angolensis* oil has a mild activity on *Staphylococcus epidermidis* and *Klebsielle pneumoneae* and a weak sensibility on *Staphylococcus aureus* CIP 4.83 and *Candida albicans* ATCC 10231. This study highlights chemical composition of the EO from *P. angolensis* and *P. quadrifolia* leaves. It shows more similarities of the chemical compositions of these two species of *Premna* and the interesting antimicrobial activity potential of *P. angolensis* EO.**

***Keywords:-*** *Premna angolensis, Premna quadrifolia, essential oil, GC-MS, Côte d'Ivoire.*

## I. INTRODUCTION

Plants are being used for the treatment of several diseases in Côte d'Ivoire, by the populations. Among the species used in traditional Ivorian medicine, many are aromatic plants that produce essential oils. *Premna angolensis* and *Premna quadrifolia* are two of these plants used in traditional medicine. The genus *Premna* comprises about 225 species, distributed mainly in the tropics and sub-tropics. It has already been classified within the family Verbenaceae (**Munir, 1984**), but has been transferred to the family Lamiaceae (**Harley et al. 2004; APG,2009 ; Olmstead 2010, 2012**). Morphologically, most species of the genus *Premna* are small trees or shrubs and rarely found as lianas (**De Kok 2013**). *Premna angolensis* is widely found in tropical Africa, from eastern Senegal to Ethiopia, Kenya, Tanzania and Angola. Small to medium-sized tree up to 21 (–33) m tall, less often shrubby; bole up to 120 cm in diameter, often curved, sometimes fluted, usually hollow **(Chase et Reveal, 2009 ; Haston et al., 2009**). In Côte d'Ivoire the bark of *P. angolensis* are used in enemas and baths to treat fever in children. In several other countries, *P. angolensis* is used in traditional medicine (**Burkill, 2000; Bolza et Keating, 1972 ; Fernandes, 2005 ; Lovett et *al*.,2006 ; Verdcourt,1992** ).

*Premna quadrifolia* is a shrub or small tree growing up to 3.5 meters tall. Tree of edges of gallery with downy leaves. Crumpled young shoots exhale a very bad odor (**Vergiat, 1970**). Several uses are to be noted, in particular to treat malaria, diarrhea, stomach disorders, headaches, coughs, tuberculosis, and infectious diseases such as leuchorrea, genital diseases, cancerous wounds, bad breath and white tongue (**Girardi et al. 2015** ; **Perry and Metzger, 1980 ; Quattrocchi , 2012** ; **Sharma et coll. 2014**). However, the EO of the leaves of these plants are not yet been biologically investigated to our knowledge in Côte d'Ivoire.

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The main objective of this work was to determine the chemical composition and antibacterial activities of EO extracted from the leaves of *P. angolensis* and *P. quadrifolia*, two aromatic plant species collected in Côte d'Ivoire.

## II. MATERIAL AND METHODS

➢ *Plant material and steam distillation*

The plant material consisted of dry leaves of *P. angolensis* and *P. quadrifolia* leaves from Prikro (7- 10' 59.999" N 4- 55' 0.001" W). The plants were identified by a botanist technician from the Swiss Center for Scientific Research in Côte d'Ivoire (CSRS, Adiopodoumé - Côte d'Ivoire) and authenticated at the National Floristic Center (CNF) of Abidjan using the existing herbarium, under the number UCJ017472 for *P. quadrifolia* and UCJ017453 for *P. angolensis*. The materials were dried at room temperature. The EO extraction was carried out in 4 hours through a steam-distillation technique. The steam distillation technique consisted of a four-compartment stainless steel device, used to extract essential oils from the plant. The boiler (capacity: 60 l ) was connected to a large tank by a stainless steel pipe. The large tank (height: 100 cm, internal diameter: 51 cm, i.e. a volume of 0.2 $m^3$) contains four grids attached to a removable rod. On the grids, the leaves were placed. From this tank, the water vapor drives the volatile compounds into a third tank (height: 100 cm, internal diameter: 41cm, ie a volume of 0.13 $m^3$) which served as a refrigerant. The EO are obtained in a fourth compartment serving as a recovery system.

➢ *GC-MS analysis :*

A GC (7890A, Agilent Technologies) instrument coupled with MS (5975C, Agilent Technologies). The liquid sample volume of 1 µl was injected to a liner with 250°C and a split ratio of 100:1. The capillary column HP-5MS was used. Oven temperature programming was as follows: 40°C (hold 5 min), then a rate of 2°C/min to 250°C; then a rate of 10°C/min to 300°C. The carrier gas helium flow was 1 mL/min. The source and transfer line of MS detector were at 230 and 280°C, respectively, while the detector voltage was 1.4 kV, and the scan range of mass-to-charge ratio of ion was 40-500.

➢ *Evaluation of in vitro antibacterial activities*

The strains used for sensitivity studies of the Essential Oils were *Staphylococcus auréus CIP 4.8* ; *Staphylococcus épidermidis CIP.53124* ; *Salmonella typhimirium SO 66* ; *Eschérichia coli ATCC 25922* ; *Klébsiella pneumoneae* ; *Pseudomonas aéruginosa ATCC 27853* ; *Bacillus subtulis ATCC 6633* ;*Candida albicans ATCC 10231* ; *Candida tropicalis ATCC 13803* et *Candida glabrata ATCC 66032* obtained from the laboratory of Suisse Center for scientific research in Côte d'Ivoire. The antibacterial activity of the EO against each bacterial strain at different concentrations was determined using the method of Berghe and Vlietinck (**Berghe et Vlietinck, 1991**).The inoculum of 1 mL was sown in a culture for 18 hours to 20 hours ($10^5$-$10^6$ UFC/mL), on Mueller-Hinton agar. After 15 min, wells were cut using Pasteur pipettes. The bottom of the wells were closed with a drop of MH agar to limit the diffusion of the oils under the agar. Then, 50 µL of the oil at different dilutions was distributed into each well. After diffusion, cultures were incubated in incubators at 37°C for 24 hours. Inhibition rings were measured with a caliper. 0.1 mL of 18 hours broth of *Escherichia coli*, *Salmonella*, *Bacillus*, *klebsiella*, *candida* was transplanted into 10 mL of MH broth and 0.3 mL for *Staphylococcus aureus* and *epidermidis*. Incubated at 37°C for 3 to 5 hours until the appearance of a slight opalescence of about $5{,}10^7$ bacteria/mL. Then 1 mL of these broths ware added to 10 mL of MH broth previously heated to 37 ° C (inoculum). Then 100 µL of the essential oil extract solution was placed in column No. 12 of the microplate, 50 µL of MH broth from column No. 11 to column No. 2 and 100 µL of MH broth in column 1. A dilution from column no. 12 to column no. 3, taking 50 µL each time, and 50 µl of the inoculum was distributed into each well of columns no. 12 to no. 2. The reading was made with the naked eyes after incubation at 37°C for 18 hours. The concentration of the last cup where there is no turbidity was also noted. The antibiotics Amphotericin B and Gentamicin were used as references.

## III. RESULTS AND DISCUSSION

➢ *Extraction yield and Chemical composition of the EO*

The EO extracted from the leaves of *P. angolensis* and *P. quadrifolia* are yellow, they have an aromatic odor with yields of 0.10 ± 0.02% and 0.40 ± 0.02% respectively. These yields, which could be attributed to the distillation technique, was calculated by the quotient of the mass of EO extracted to the mass of the distilled plant material, are good in view of several yields published on plants harvested in Côte d'Ivoire. It's the case for the trunk bark of *Cleitopholis patens* (0.18-0.23%), (**Ouattara, 2012**) and for the leaves of *Melanthera scandens* (0.012), (**Konan, 2015**). The genus *Premna* is not widely known to be high in EO content. Nonetheless, previous studies have reported the EO content to be in a range of 0.056 to 0.102% in some species of *Premna* ( **Narayan et Muthana, 1953; Teai et al. 1998; Chanotiya et al. 2009; Rahman et al. 2011; Sadashiva et al. 2013; Adjalian et al. 2015**). The identification of their constituents was carried out by GC-MS. The retention indices were determined from the retention times **(Kovats, 1958; IUPAC, 1997**). Twenty-seven (27) compounds were identified in the EO of the leaves of *P. angolensis*, about 99.43% of total composition (Table1).The chemical composition of the EO contains 71.90% of hydrocarbon sesquiterpenes, 14.16% of oxygenated compounds, 13.22% of monoterpenes and 0.15% of alkenes. The main compounds are β-caryophyllene (33.07%) and humulene (10.78%). These results are quite similar to those found in the literature. Adjalian and al. (2015) analyzed by GC-MS, the EO of the leaves of *P. angolensis*. They identified twenty-nine (29) compounds of which the most predominant are the hydrocarbon sesquiterpenes (26.6%) and oxygenated sesquiterpenes (20%). The major compounds were octen-3-ol (28%) and (E) -β-caryophyllene (13.5%).

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With regard to its total chemical composition, the essential oil from the leaves of *P. quadrifolia* we identified 36 compounds, about 99.38% (Table 2). Mainly consisted of hydrocarbon sesquiterpenes (89.15%), oxygenated compounds (8.61%) and monoterpenes (1.61%). The main compounds are α-bulnesene (23.33%), germacrene D (18.83%) and caryophyllene (18.06%). Adjalian et al. (2015) had performed a GC-MS analysis of the EO of the leaves of this plant which gave similar results. They revealed forty-two (42) compounds, the most predominant of which are sesquiterpenes (65.5%). The predominant compounds are β-elemene (21%) and β-caryophyllene (13.1%) (Adjalian et al. 2015).

According to these results, several compound well distributed in the two species of *Premna* studied in varying concentrations. But they are different major compounds.

| Number | Compounds | RT (min) | RI | m/z | % of total |
|---|---|---|---|---|---|
| **1** | α-Pinene | 12.8 | 927 | 136 | 0.24 |
| **2** | Sabinene | 15.5 | 969 | 136 | 1.68 |
| **3** | β-Myrcene | 16.9 | 991 | 136 | 6.14 |
| **4** | D-Limonene | 19.2 | 1025 | 136 | 4.82 |
| **5** | γ-terpinene | 20.9 | 1048 | 136 | 0.34 |
| **6** | α-Cubebene | 41.4 | 1345 | 204 | 0.47 |
| **7** | Copaene | 43.0 | 1370 | 204 | 5.60 |
| **8** | β-Elemene | 44.1 | 1387 | 204 | 7.64 |
| **9** | **(E)-β-Caryophyllene** | **45.6** | **1412** | **204** | **33.07** |
| **10** | **Humulene** | **47.7** | **1446** | **204** | **10.78** |
| **11** | 1,8-Nonadiene, 2-methyl-5,7-dimethylene- | 48.1 | 1453 | 161 | 0.15 |
| **12** | (Z)-β-Caryophyllene | 48.3 | 1457 | 204 | 0.42 |
| **13** | Germacrene D | 49.4 | 1475 | 204 | 7.54 |
| **14** | (+)-Eremophilene | 50.3 | 1489 | 204 | 0.83 |
| **15** | α-Muurolene | 51.5 | 1509 | 204 | 1.13 |
| **16** | Cadina-1(10),4-diene | 52.1 | 1519 | 204 | 3.28 |
| **17** | Germacrene B | 53.8 | 1549 | 204 | 0.36 |
| **18** | beta-Farnesene | 54.6 | 1564 | 204 | 0.78 |
| **19** | Caryophyllene oxide | 55.3 | 1584 | 222 | 6.89 |
| **20** | 6-epi-Cubenol | 56.8 | 1601 | 222 | 1.29 |
| **21** | Epi-Cadinol | 58.7 | 1635 | 222 | 0.58 |
| **22** | Selin-11-en-4-ol | 59.1 | 1643 | 222 | 0.26 |
| **23** | α-Eudesmol | 59.4 | 1649 | 222 | 0.35 |
| **24** | α-Cadinol | 60.2 | 1663 | 222 | 0.40 |
| **25** | Phytone | 69.7 | 1845 | 250 | 1.22 |
| **26** | Phytol | 82.3 | 2114 | 296 | 2.80 |
| **27** | Phytolacetate | 83.5 | 2200 | 338 | 0.37 |
| Monoterpenes | | | | | 13.22 |
| Sesquiterpenes | | | | | 71.90 |
| Oxygenated Compounds | | | | | 14.16 |
| Others | | | | | 0.15 |
| Total | | | | | 99.43 |

RT : RetentionTime ; RI : Retention Indice

Table 1:- Constituents identified in the EO of *P. angolensis* leaves

| Number | Compounds | RT (min) | RI | m/z | % of total |
|---|---|---|---|---|---|
| **1** | α-Pinene | 12.7 | 927 | 136 | 0.40 |
| **2** | Sabinene | 15.5 | 969 | 136 | 0.78 |
| **3** | D-Limonene | 19.2 | 1025 | 136 | 0.43 |
| **4** | α-Cubebene | 41.4 | 1345 | 204 | 0.06 |
| **5** | Copaene | 42.7 | 1366, | 204 | 0.23 |
| **6** | α-Ylangene | 43,0 | 1370 | 204 | 1.38 |
| **7** | β-Elemene | 43.7 | 1381 | 204 | 0.13 |
| **8** | β-Cubebene | 44.1 | 1388 | 204 | 5.39 |
| **9** | **Caryophyllene** | **45.6** | **1412** | **204** | **18.06** |
| **10** | (+)-Calarene | 46.2 | 1422 | 204 | 0.19 |
| **11** | α-Guaiene | **46.9** | **1434** | **204** | **13.48** |

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| | | | | | |
|---|---|---|---|---|---|
| **12** | Humulene | 47.7 | 1446 | 204 | 4.16 |
| **13** | (Z)-β-Caryophyllene | 48.3 | 1457 | 204 | 0.23 |
| **14** | (E)-Dodec-2-enol | 48.7 | 1462 | 189 | 0.02 |
| **15** | **Germacrene D** | **49.4** | **1475** | **204** | **18.83** |
| **16** | (+)-Eremophilene | 50.3 | 1488 | 204 | 1.62 |
| **17** | **α-Bulnesene** | **51,0** | **1500** | **204** | **23.33** |
| **18** | Cadina-1(10),4-diene | 52.1 | 1520 | 204 | 0.82 |
| **19** | Germacrene B | 53.8 | 1549 | 204 | 1.22 |
| **20** | palustrol | 54.9 | 1569 | 222 | 0.24 |
| **21** | Caryophyllene oxide | 55.3 | 1575 | 222 | 1.88 |
| **22** | β-Elemenone trans | 55.9 | 1586 | 222 | 0.12 |
| **23** | α-Guaiol | 56.4 | 1594 | 222 | 0.39 |
| **24** | 6-epi-Cubenol | 56.8 | 1602 | 222 | 0.52 |
| **25** | 1,10-di-epi-Cubenol | 57.2 | 1609 | 222 | 0.13 |
| **26** | 4(15)-Eudesmen-6-ol | 57.4 | 1613 | 222 | 0.16 |
| **27** | (+)-epi-Cubenol | 58.05 | 1624 | 222 | 0.35 |
| **28** | Cubenol | 58.5 | 1633 | 222 | 0.86 |
| **29** | Selin-11-en-4-ol | 59.1 | 1643 | 222 | 0.19 |
| **30** | epi α-Cadinol | 59.3 | 1648 | 222 | 0.81 |
| **31** | α-Cadinol | 60.1 | 1662 | 222 | 0.47 |
| **32** | Germacra-4(15),5,10(14)-trien-1α-ol | 61.1 | 1680 | 222 | 0.14 |
| **33** | (E,E)-Farnesol | 63.0 | 1715 | 222 | 0.1 |
| **34** | Oplopanone | 63.9 | 1732 | 238 | 0.09 |
| **35** | Phytol | 82.3 | 2115 | 297 | 2.03 |
| **36** | Phytolacetate | 83.5 | 2141 | 123 | 0.13 |
| Monoterpenes | | | | | 1.61% |
| Sesquiterpenes | | | | | 89.15% |
| Oxygenated compounds | | | | | 8.61% |
| Total | | | | | 99.38% |

RT : Retention Time ; RI : Retention Indice

Table 2:- Constituents identified in the EO of *P. quadrifolia* leaves.

➢ *Antibacterial potential*

After the incubation, there was a zone of inhibited growth around the well, the size of which was related to the antimicrobial capacity of the substance. Results (table 3), showed that the EO of the leaves of *P. quadrifolia* has no activity on bacterial strains and fungi. As for the EO of the leaves of *P. angolensis*, it has a weak activity on *Staphylococcus aureus* CIP 4.83 and *Candida albicans* ATCC 10231. It has a mild activity on *Staphylococcus epidermidis* and *Klebsielle pneumoneae.* Indeed the activity is considered nonexistent for a diameter of inhibition (d.i.) less than or equal to 8 mm; weak for d.i. between 8 and 14 mm, mild for d.i. between 14 and 20 mm; strong for d.i. greater than or equal to 20 mm (**Berghe et Vlietinck, 1991**). The difference in sensitivity of EO with respect to the strains despite an almost similar composition could be due to the major compounds which are different.

| | d.i of EO PA (mm) | d.i of PQ EO(mm) | d.i Gent.(reference)(mm) |
|---|---|---|---|
| *Bacillus subtulis* ATCC 6633 | 00 | 00 | 34 |
| *Staphylococcus aureus* CIP 4.83 | 10 | 00 | 30 |
| *Staphylococcus epidermidis* CIP.53124 | 15 | 08 | 30 |
| *Pseudomonas aeruginosa* ATCC 27853 | 00 | 00 | 30 |
| *Salmonella typhimirium* SO 66 | 00 | 00 | 28 |
| *Escherichia coli* ATCC 25922 | 00 | 00 | 28 |
| *Klebsielle blse* | 15 | 08 | 35 |
| | | | Amphotericin B (reference) |
| *Candida albicans* ATCC 10231 | 09 | 00 | 35 |
| *Candida tropicalis* ATCC 13803 | 08 | 00 | 30 |
| *Candida glabrata* ATCC 66032 | 08 | 00 | 35 |

d.i : diameter of inhibition

Table 3:- Diameter of inhibition of EO on bacteria and fungi

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The MIC and BMC of the EO of the leaves of *P. angolensis* are determined from the inhibition diameter greater than or equal to 11mm (Table 4).

| | MIC mg/ml | MBC mg/ml | MBC/MIC | Effect of the EO |
|---|---|---|---|---|
| *Klebsiella pneumoneae* | 3.75 | 30 | 8 | bacteriostatic |
| *Stahylococcus epidermidis* | 3.75 | 30 | 8 | bacteriostatic |

MIC: Minimal Inhibitory Concentration; MBC : Minimum Bactericidal Concentration

Table 4:- Minimum inhibitory and bactericidal concentration of EO of the leaves of *P. angolensis*

The EO of the leaves of *P. angolensis* is bacteriostatic. It therefore inhibits the multiplication of bacteria without killing them. By inhibiting bacteria, it could work with the immune system to remove the bacteria from the body.

## IV. CONCLUSION

This study allowed us to identify the composition by GC-SM and to evaluate the bacterial activities of EO from the leaves of two *Premna* from Côte d'Ivoire. EO of the leaves of *P. angolensis* contains 71.90% of hydrocarbon sesquiterpenes, 14.16% of oxygenated compounds, 13.22% of monoterpenes and 0.15% of alkenes. The main compounds are β-caryophyllene (33.07%) and humulene (10.78%). And EO from the leaves of *P. quadrifolia* contains hydrocarbon sesquiterpenes (89.15%), oxygenated compounds (8.61%) and monoterpenes (1.61%). The major compounds are α-bulnesene (23.33%), germacrene D (18.83%) and caryophyllene (18.06%). The composition of the two species are almost identical.

Antimicrobial tests showed that EO of the leaves of *P. angolensis* oil has a mild activity on *Staphylococcus epidermidis* and *Klebsielle pneumoneae* and a weak sensibility on *Staphylococcus aureus* CIP 4.83 and *Candida albicans* ATCC 10231. So these activities would justify the use of extract of *P. angolensis* for treatment by the populations. That of *P.quadrifolia* has no activity on bacterial strains and fungi.

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- *Statement of Conflict of Interest and Source of Funding*

The Authors declare absence of any conflicts of interest. In addition, no funding was received.

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