ISOLATION, CHARACTERIZATION AND RADICAL SCAVENGING SCREENING OF LIGNANS AND ALKALOIDS FROM CALONCOBA GLAUA (FLACOURTIACEAE).

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Abstract

Caloncoba glauca, a plant from Central Africa (Cameroun, Gabon), it’s used for healing skin diseases. The aim of this work is to carry out the phytochemical study of this plant as well as to evaluate the free-radical scavenging activity of its constituents. Repeated silica gel column chromatography followed by Sephadex LH-20 were used to isolate four compounds and their structures were elucidated using spectroscopic analysis, and comparison with published data. The free-radical scavenging assay on a microplate using DPPH was carried out to evaluate the antioxidant activity of the samples. These spectroscopic data allowed us to identify a lignan (sesamin), an alkaloid (dictamin) and a triterpene (lupeol). Lignans and alkaloids have been isolated for the first time from this plant species. The antiradical activity has been highlighted by the use of bio autographic method and disclosed to DDPH. As results, the ethyl acetate extract, sesamin and dictamin revealed antiradical activity. This work reveals the antioxidant activity of sesamin and dictamin. We are currently identifying other compounds and investigating cytotoxicity and other pharmacological activities with the anticancerous potential.

Introduction:

The genus Caloncoba belongs to the economically and medicinally important family Flacourtiaceae. This family is well known for its medicinal Chaulmoogra oil, which has been used for a very long time for the treatment of skin diseases and especially for leprosy, and as an ointment for tuberculosis patients (Ziegler, et al., 2002). Caloncoba glauca (P. Beauv.) Gilg is a small to 15 m tall tree found in tropical Africa (Burkill, 1994). The leaves of this plant are used in traditional medicine as purgative, the fruits serve as poison for fishing, the stem bark, leaves and fruits are used against inflammations and skin diseases (Mpeta, et al., 2012). Previous work in this plant show the presence of alkaloids, lignans and their antimicrobial activity (Agbo, et al., 2017), also cycloartanes and friedelanes (Giner-Pons, et al., 1992; Giner, 1993; Mpeta, et al., 2012; Simo et al., 2012). In the course of our ongoing search for potent bioactive compounds from Gabonese medicinal plants, and in order to rationalize the medicinal utilization of Caloncoba glauca, we investigated the chemical constituents of the ethyl acetate extract from the stem barks, as well as their radical scavenging activity.
Materials and Methods:-
The stem barks of *C. glauca* were cut, dried at room temperature and then ground to give a powder (2.5 kg) which was macerated in ethanol (10 L x 3). After filtration and removal of the solvent using rotary evaporator, 150 g of the crude extract was obtained. 140 g of this extract was re-extracted using ethyl acetate (EtOAc) to afford 78.7 g of the ethyl acetate extract. Part of the EtOAc extract (70 g) was subjected to silica gel column chromatography eluted with gradient polarity of hexane, EtOAc, and methanol (MeOH) to give five fractions (F<sub>1</sub>-F<sub>5</sub>). Fraction F<sub>2</sub> (28.5 g) (hexane-EtOAc, 4:1) was rechromatographed using similar mobile phase as described above to afford four compounds. Silica gel 60 F<sub>254</sub> (70–230 and 230–240 mesh; Merck; Darmstadt, Germany) and Sephadex LH-20 were used for Column Chromatography while precoated silica gel Kieselgel 60 F<sub>254</sub> plates (0.25 mm thick) were used for analytical thin layer chromatography. The structures of the isolated compounds were determined by interpretation of their spectroscopic data (IR, NMR and MS) and comparison of these data with those from the literature. The free radical scavenging property of the EtOAc extract and isolated compounds was evaluated using TLC plates eluted with hexane-EtOAc (4:1) and sprayed with a 0.2% methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Wako, Japan)

Results and Discussion:-
Four compounds were isolated from the EtOAc extract of the stem barks of *C.glauca*. Three of these compounds (Fig. 1) were identified and belong to three different classes of secondary metabolites mainly lignans, terpenoids and alkaloids. These compounds were identified as sesamin (1), lupeol (2) and dictamin (3), respectively.

The antioxidant activity of a compound is based on the capacity of the latter has trap the free radicals. The EtOAc extract and compounds 1-3 were screened for their radical scavenging using the free stable radical DPPH. The reduction of DPPH (purple) to the corresponding hydrazine (yellow) is a classic, simple and fast method for evaluating radical scavenging activity (Brand-williams , et al., 1995). Observation of the yellow spots on TLC plate (Fig. 2) indicated radical scavenging activity for the extract and compounds 1 and 3. Compound 2 did not show activity and an attempt to explain that could be the absence of aromatic rings in its structure.
Conclusion:

The genus *Caloncoba* has been reported to be mainly rich of triterpenoids. The present study exhibited in addition to triterpenoids, the presence of lignan and alkaloid in *C. glauca*. Moreover this plant species appeared as a potential source of radical scavenging compounds. The results herein described indicate that *C. glauca* could be considered as a valuable source of lead compounds for drug discovery.

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References:

1. Agbo, A.A J.B.P., Mpetga, S.D J., Bikanga, R., Tchuenguem, R.T., Tsafack, N.R.B., Awouafack, M.D., Dzoyem, J.P., Ito, T., Morita, H., Tané, P. (2017). «A New Benzophenanthridine Alkaloid from *Caloncoba glauca*.» Natural Product Communications., 12 (3): 367-368.
2. Brand-Williams, W., Cuvelier, M.E., Berset, C. (1995). «Use of a free radical method to evaluate antioxidant activity.» Food Science and Technology., 28 (1): 25-30.
3. Burkill, H M. (1994). The useful plants of west tropical Africa. Vol. 2. Kew Royal Botanic Gardens. England.
4. Giner, R M., Gray A.I., Gibbons S., Waterman P.G. (1993). «Friedelane triterpenes from the stem bark of *Caloncoba glauca*.» Phytochemistry., 33 (1): 237-239.
5. Mpetga, J.D.S., Tene, M., Wabo, K.H., Li, S.F., Kong, L.M., He, H.P., Hao, X.J, Tané, P. (2012). «Cytotoxic cycloartanes from the fruits of *Caloncoba glauca*.» Phytochemistry Letters., 5 (1): 183-187.
6. Ziegler, H.L., Staerk, D., Christiensen J., Olsen C.E., Sittie, A.A., Jarosweski, J.W. (2002). «New dammarane and malabaricane triterpenes from *Caloncoba echinata*.» Journal of Natural Products., 65: 1764-1765.