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IDENTIFICATION OF COMPOUNDS PRESENT IN THE DICHLOROMETHANE FRACTION OF YOUNG UNOPENED LEAVES OF PILIOSTIGMA THONNINGII BY LC-MS

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ABSTRACT
Piliostigma thonningii (Schumach Mile-Redh) is a species of the Fabaceae family. In Côte d'Ivoire, traditional practitioners use the young, unfolded leaves of this species to treat a number of ailments. The aim of this study was to determine the compounds present in the dichloromethane fraction of young, unopened leaves of *Piliostigma thonningii*. The successive fractionation process of the hydroethanol extract resulted in a fraction with dichloromethane. LC-MS analysis identified six (6) compounds in this fraction. These analyses revealed that the young, unopened leaves of *Piliostigma thonningii* contain enormous unidentified chemical compounds.

KEYWORDS: *Piliostigma thonningii*, Fabaceae, LC-MS, Pathology.

1. INTRODUCTION
Piliostigma thonningii is one of the many plants still little explored in the abundant flora of Côte d'Ivoire, a veritable treasure trove of bioactive molecules. This leguminous plant belongs to the Fabaceae family and comprises around 133 species. The roots and twigs are used to treat dysentery, fever, respiratory ailments, snake bites, hookworm and skin infections **(Kwaji *et al.*, 2010)**. In addition, fresh leaves are used to treat ulcers **(Ukwuani *et al.*, 2012)** and malaria **(Madara *et al.*, 2010).** According to **(Salawu *et al.*, 2007)**, a hot infusion of the leaves helps to reduce fever, dental pain and diarrhoea. These leaves are characterised by their anti-inflammatory and antibacterial **(Pousset, 2006)**, antifungal **(Agban *et al.*, 2013)** and antioxidant **(Dieng *et al.*, 2017)** properties. They are also known for their antipyretic, analgesic and antimalarial effects **(Salawu *et al.*, 2007).** The young, unopened leaves of *Piliostigma thonningii* are traditionally used by traditional medicine practitioners in Côte d'Ivoire to treat certain ailments. The aim of this study was to identify the phytocompounds present in these young leaves using the LC-MS method.

2. MATERIALS AND METHODS
2.1 Plant material
Young, unopened leaves of *Piliostigma thonningii* were collected before sunrise in May 2018 in Bouaké (6047'18.762'' North and 5015'25.9992'' West) in central Côte d'Ivoire and identified by Mr Amani N'Guessan, botanist at the Institut National Polytechnique Félix HOUPHOUËT-BOIGNY (INP-HB) in Yamoussoukro.

2.2. Matériel technique
Le Mass Hunter est un logiciel d'analyse de données spectrométriques (CLHP-ESI-MS) fournies par l'appareil Quadripôle Time Of Flight (Q-TOF) de marque Agilent, Q-TOF 6200, série TOF/6500 séries Q-TOF B.08.00 (B8058.0).

3. METHODS
3.1. Preparation of the samples
The leaves were dried in the shade at room temperature in the laboratory (26-30°C) for 14 days and then ground. The resulting powders were sieved using a 0.4 µm mesh sieve and stored in coloured jars at 4°C, protected from light and humidity, until further use.

3.2. Plant material extraction and fractionation procedure

Plant material was extracted in an ethanol/water mixture (70/30) according to the method described by **(Kassi *et al.*, 2014)**. After extraction, a 25 g mass of the hydroethanol extract was dissolved in 250 mL of water at 60°C and fractionated successively with 2 x 250 mL of hexane and dichloromethane. The resulting aqueous phase was evaporated to remove the water and oven dried at 50°C for 4 h. The different organic phases obtained were dried separately over anhydrous sodium sulphate. After filtration and removal of the solvents under reduced pressure, the hexane and dichloromethane fractions were obtained. The dichloromethane fraction was used for HPLC-ESI-Q-TOF-MS analysis.

4. Analysis of LC-MS

To carry out the HPLC-ESI-Q-TOF-MS analysis of the fractions, we used an Agilent LC-MS system combined with an Agilent 1260 Infinity HPLC system coupled to a mass spectrometer (Agilent 6530 Q-TOF-MS) equipped with an ESI source. Analyses were performed in positive mode. A Sunfire ® C18 Waters analytical column 150 mm long, 2.1 mm in diameter and 3.5 µm in diameter was used with a flow rate of 250 µL/min and a two-way linear gradient : Lane A (95-0 % H_2O + 0.1 % formic acid), Lane B (5-100 % ACN) for 40 minutes. A linear gradient was used in the following proportions : 0-5 % B (0-5 min), 5-95 % B (5-15 min), 95-100 % B (15-25 min), 100 % B (25-30 min), 100-0 % B (30-32 min) and 0 % B (32-41 min). The sample injection volume was set at 5 µL. Data analysis was carried out using Agilent MassHunter Workstation software.

5. RESULTS AND DISCUSSION

5.1. Derivative analysis of the dichloromethane fraction

A derivative analysis carried out on the dichloromethane fraction of young unopened leaves of *Piliostigma thonningii* detected peaks associated with molecules, whether previously identified or not. To achieve this result, the raw HPLC-ESI-MS data were processed automatically using Mass Hunter software. This presents a list of data that is graphically translated into peaks, each representing a compound contained in the dichloromethane fraction. **Fig. 1** shows the overall chromatographic profile of all the ions detected in positive mode.

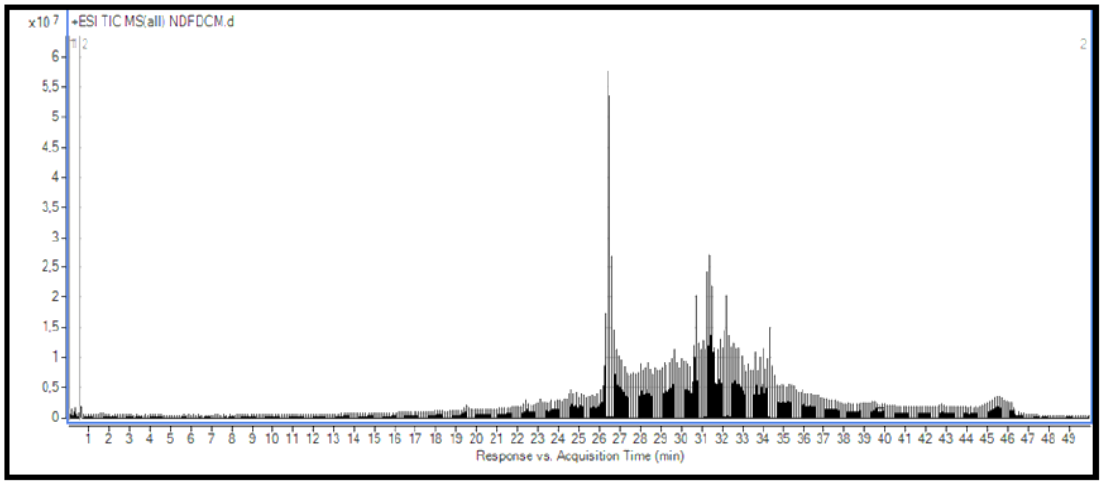

Fig. 1: Total chromatographic profile by electrospray ionisation mass spectrometry of the dichloromethane fraction of young unopened leaves of *Piliostigma thonningii*.

5.2. HPLC-ESI-Q-TOF-MS analysis of the dichloromethane fraction

HPLC-ESI-Q-TOF-MS analysis of the dichloromethane fraction of young, unopened leaves of *Piliostigma thonningii* was used to detect certain compounds. These include phenolic acids, alkaloids, sterols, terpenes and flavonoids. The coexistence of phenolic compounds is shown by **peaks 15, 17, 18, 19, 20, 21, 22 and 23**, which justifies the typical fragment mode observed. Flavonoids are identified by **peak 1**, while sterols, terpenes and alkaloids are highlighted by **peaks 22 and 23** respectively, justifying the fragmentation mode. The intense **peak 2**, with a retention time of 26, is consistent with a compound whose fragments do not correspond to the fragmentation mode of the compounds identified. The structure of the compounds identified in the dichloromethane fraction is shown in **Fig. 2.**

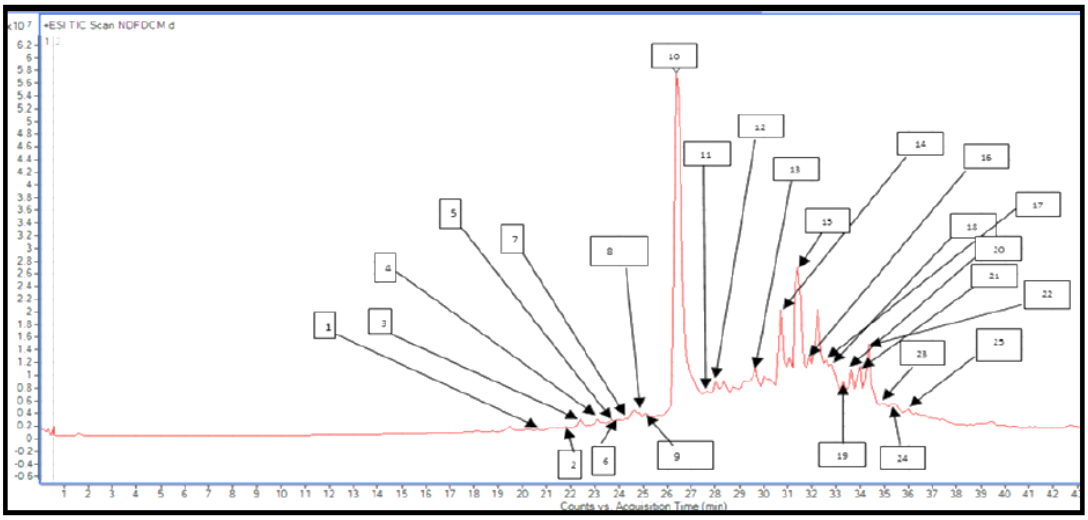

Fig. 2: MS chromatographic profile of the dichloromethane fraction of young unopened leaves of *Piliostigma thonningii*.

5.3. UV analysis

The chromatographic profiles of the HPLC-ESI-Q-TOF analysis of the dichloromethane fraction of young unopened leaves of *Piliostigma thonningii* are shown in **Fig. 3 and 4**. The intense chromatographic profiles indicate that the fraction contains several different groups of secondary metabolites, some of which are present in significant quantities. UV visualisation at wavelengths of λ_{max} 254 and 280 nm shows significant peaks in the retention time intervals 16-40 min and 27-29 min **(Fig. 3 and 4)**. Observation of these compounds at these wavelengths assumes that they have at least two conjugated double bonds (C=C-C=C or C=C-C=O) in their carbon chain **(Qu *et al.*, 2007**).

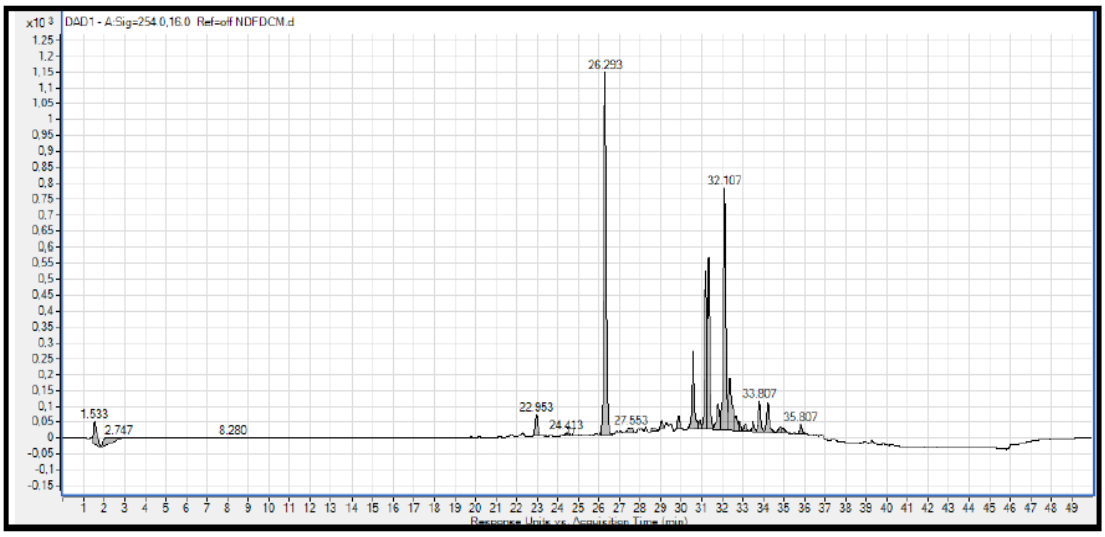

Fig. 3: UV chromatographic profile at 254 nm of the dichloromethane fraction of young unopened leaves of *Piliostigma thonningii*.

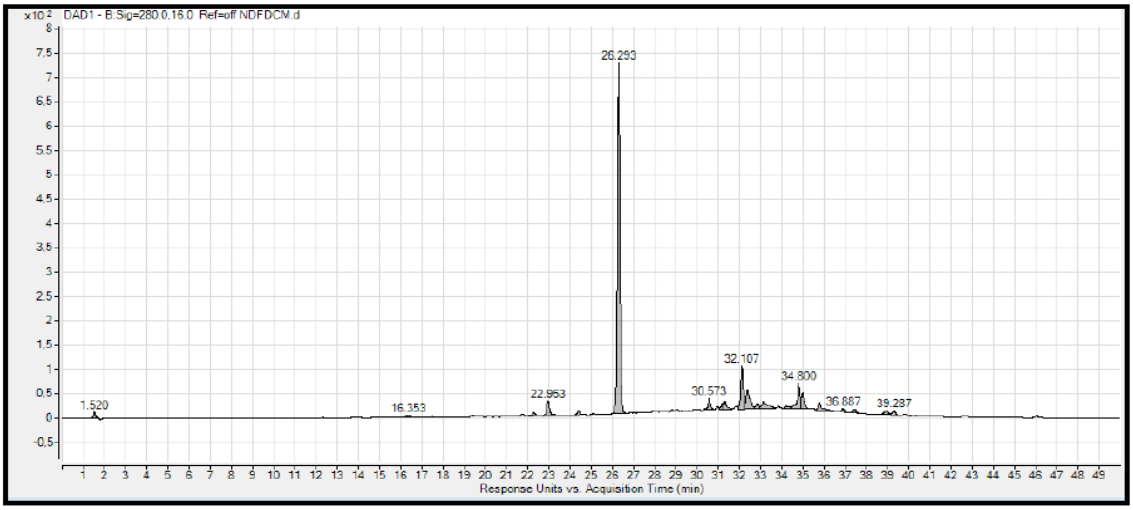

Fig. 4: UV chromatographic profile at 280 nm of the dichloromethane fraction of young unopened leaves of *Piliostigma thonningii*.

5.4. LC-MS analysis of the dichloromethane fraction

HPLC-ESI-Q-TOF-MS analysis of the dichloromethane fraction revealed the coexistence of several phytocompounds represented on the chromatogram in **Fig.1**. The structural characteristics of these compounds are shown in **Table 1**. The presence of these phytocompounds is indicated by several **peaks (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, and 24)**.

Peak 4 highlights the presence of quercetin. It is characterised by the molecular ion m/z 303 $[M+H]^+$ and daughter ions of m/z = 285 $[M+H-OH]^+$; m/z = 153 $[M+H-C_8H_5O_3]^+$ which is the most abundant fragment ; m/z = 245 $[M+H-C_2HO_2]^+$; m/z = 109 $[M+H-C_9H_5O_5]^+$; m/z = 137 $[M+H-C_8H_7O_4]^+$; m/z = 209; m/z = 201; m/z = 184; m/z = 173 and m/z = 137 **(Fig. 5).** quercetin has been isolated from flowers of Vernonia galamensis **(Keïta *et al.*, 2016)**. The fragmentation pattern of quercetin is shown in **Fig. 6**.

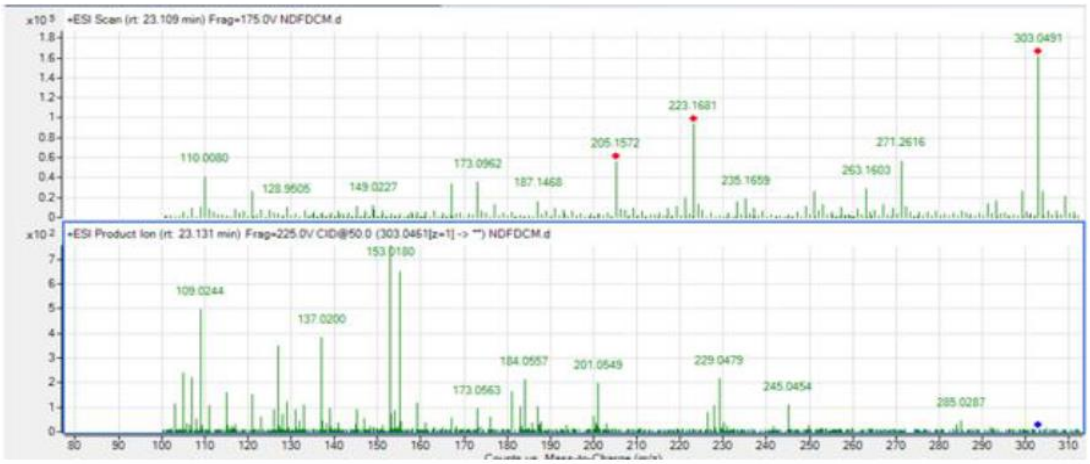

Fig. 5: MS and MS/MS mass spectra of peak 4.

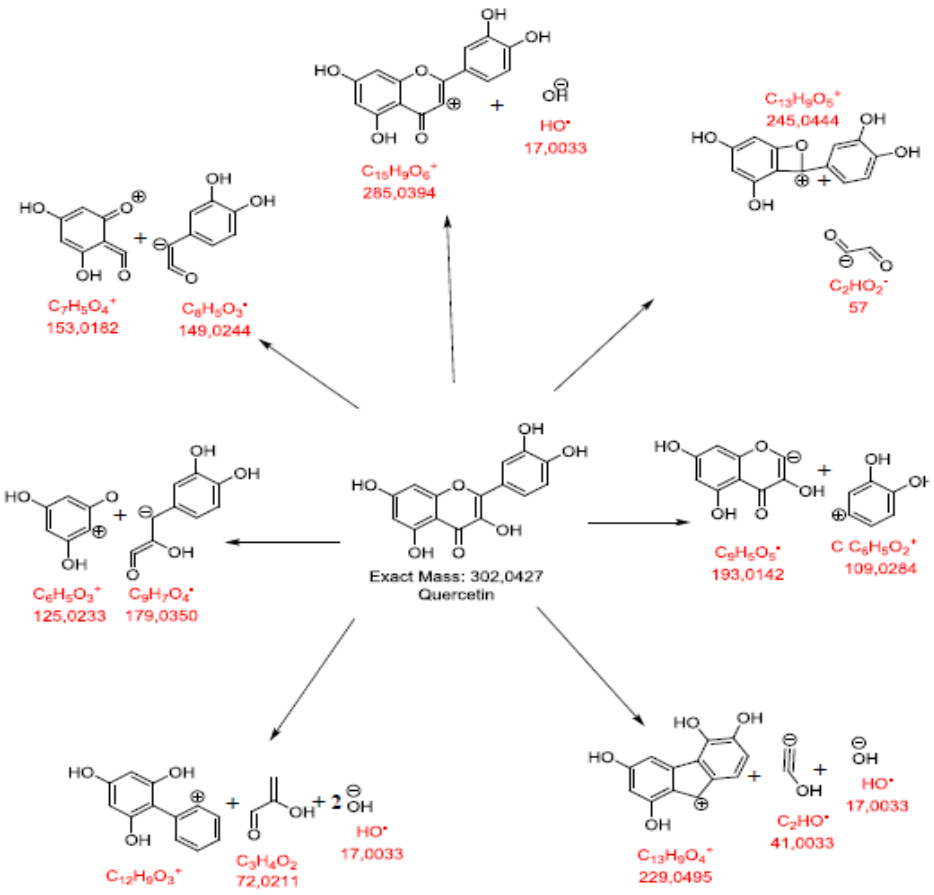

Fig. 6: Diagram of the fragmentation of quercetin.

At **peak 15,** we identified the following compound en-16-α-hydroxykauran-19-oic acid with a molecular ion m/z 321 $[M+H]^+$ and daughter ions of m/z = 297, m/z = 267, m/z = 223, m/z = 177, m/z = 159, m/z = 145, m/z = 135, m/z = 121 and m/z = 107 **(Fig. 7).** These compounds have been identified from the leaves of *Piliostigma thonningii* **(Afolayan *et al.*, 2018; Martin *et al.*, 1997)**. This compound is a mixture of three isomers; their fragmentation pattern is shown in **Fig. 8, 9 and 10.**

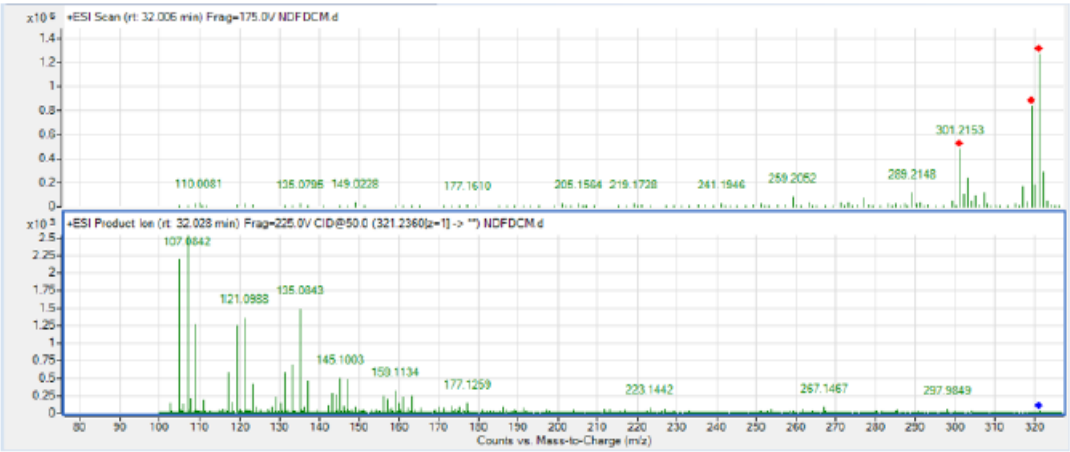

Fig. 7: MS and MS/MS mass spectra of peak 15.

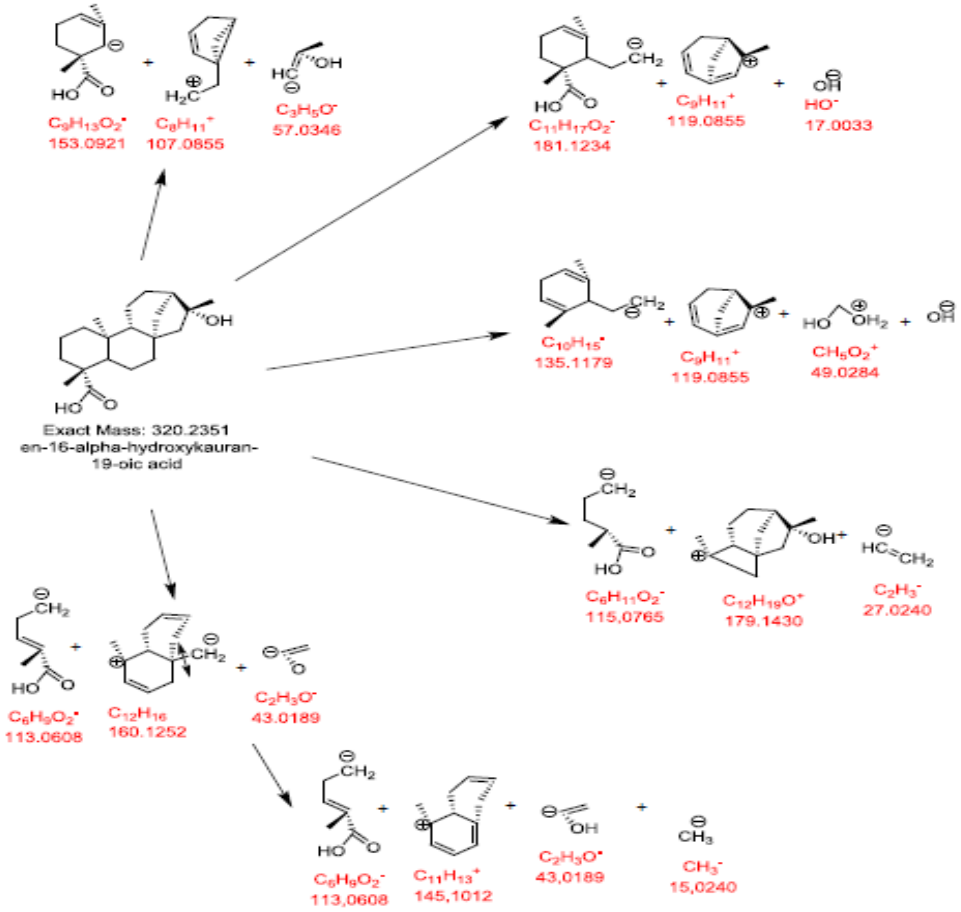

Fig. 8: Diagram of the fragmentation of en-16-α-hydroxykauran-19-oic acid.

Peaks (19) show the presence of the compounds kaur-16-en-19-oic acid and trans-communic acid, with a molecular ion m/z = 303 $[M+H]^+$ and daughter ions of m/z = 107, m/z = 119, m/z = 133, m/z = 147, m/z = 163, m/z = 173, m/z = 187, m/z = 201, m/z = 215, m/z = 233, m/z = 257 and m/z = 285 **(Fig. 11).** Kaur-16-en-19-oic acid has been identified in the leaves of Polyalthia sclerophylla **(Saepou *et al.*, 2010)** and trans-communic acid was identified by Noguera et al in 2014 **(Noguera *et al.*, 2014).** This compound is a mixture of two isomers, and their fragmentation pattern is shown in **Fig. 12 and 13**.

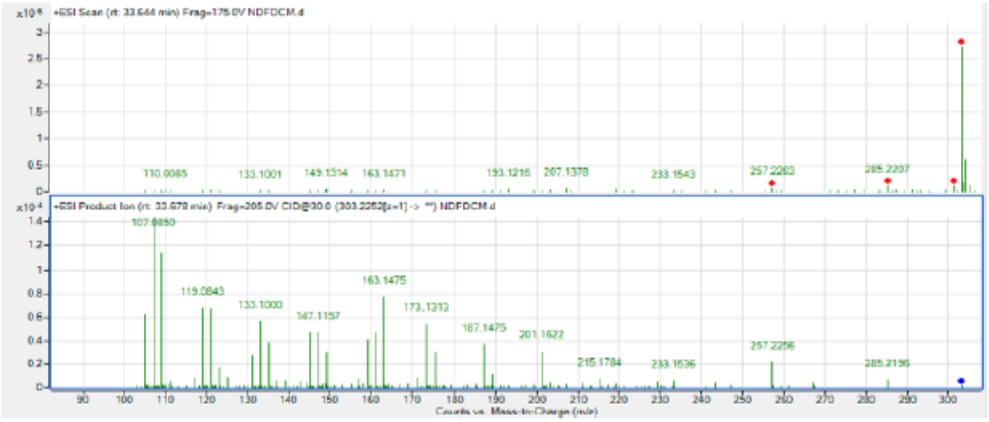

Fig. 11: Fragmentation spectra of peak 19.

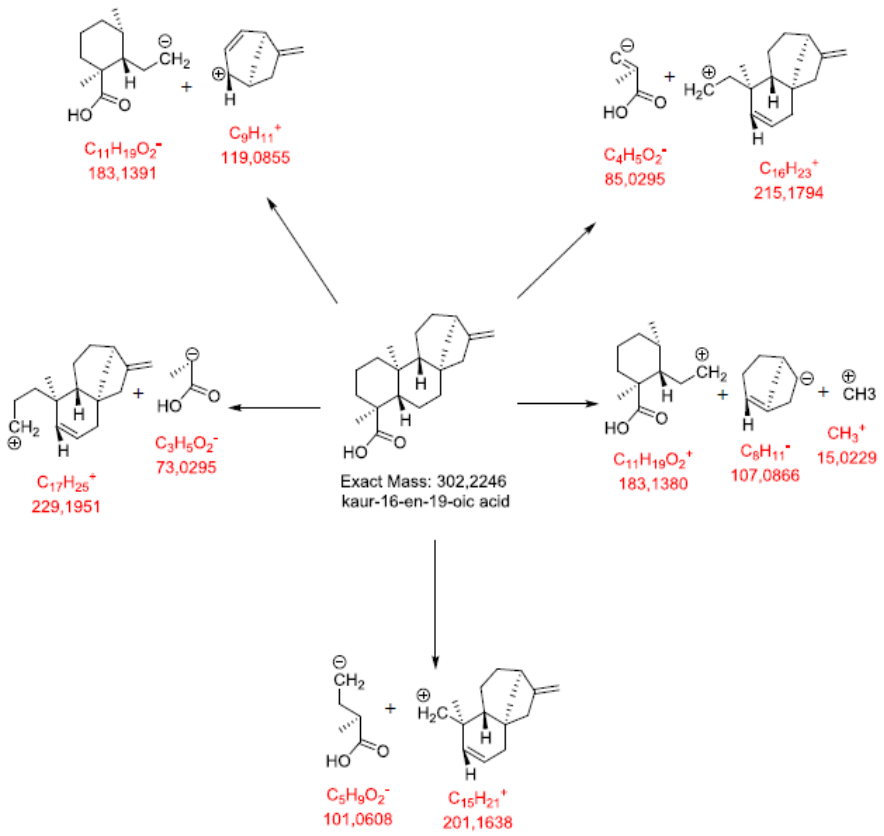

Fig. 12: Diagram of the fragmentation of kaur-16-en-oic acid.

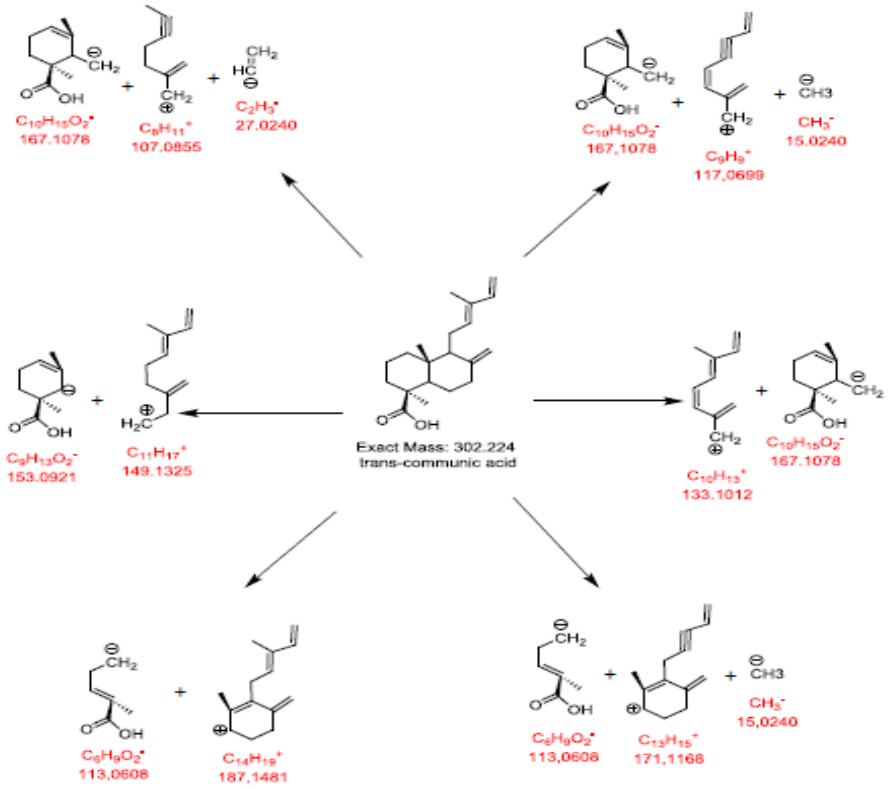

Fig. 13: Diagram of the fragmentation of trans-communic acid.

Peaks 23 highlight the presence of the anticopalic acid compound, with a molecular ion m/z 305 $[M+H]^+$ and daughter ions of m/z = 201, m/z = 182, m/z = 159, m/z = 135, m/z =121 and m/z = 107 $[M+H-C_{11}H_{15}O_2-CH_3]^+$ which is the most abundant fragment **(Fig. 14)** . This compound was identified in leaves by Afolayan **(Afolayan *et al.*, 2018)**. The fragmentation pattern of anticopalic acid is shown in **Fig.15**.

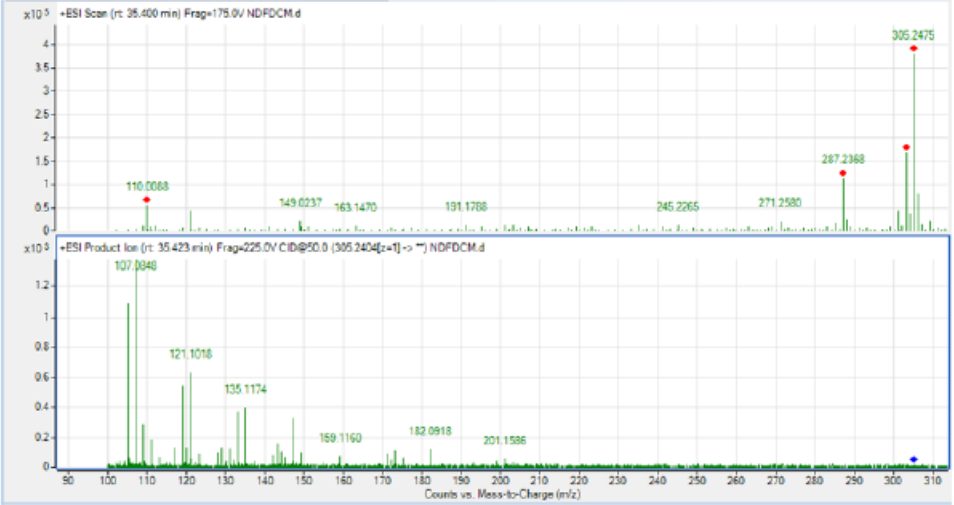

Fig. 14: Fragmentation spectrum of peak 23.

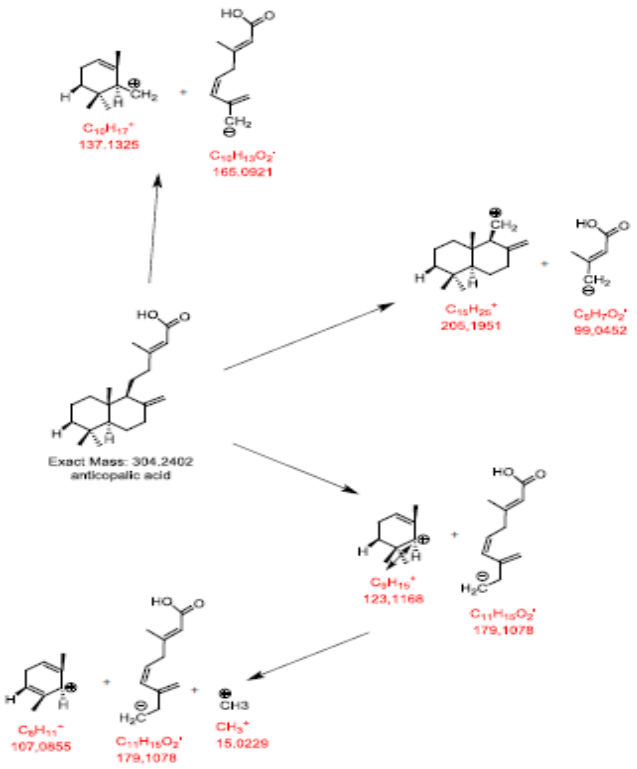

Fig. 15: Diagram of the fragmentation of anticopalic acid.

Peak 24 revealed the oleic acid amide. It is characterised by a molecular ion m/z 282 $[M+H]^+$ and daughter ions m/z 139 $[M+H-C_8H_{16}NO]^+$ which represents the most abundant fragment, m/z = 271, m/z = 254, m/z = 240, m/z = 226, m/z = 205, m/z = 180, m/z = 105, m/z = 100 **(Fig. 16).** This compound has been identified in the leaves of *Piliostigma thonningii* **(Igwe and Nwamezie, 2016)**. The fragmentation pattern of the oleic acid amide is shown in **Fig. 17.**

Fig. 16: Fragmentation spectra of peak 24.

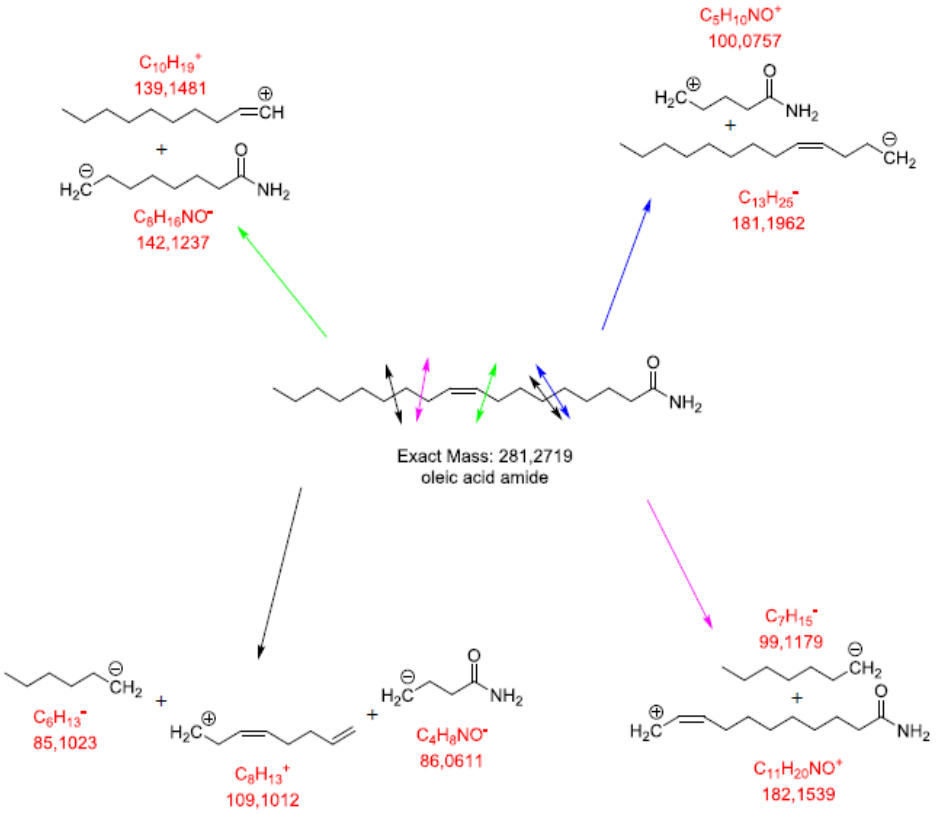

Fig 17: Diagram of the fragmentation of the oleic acid amide.

Table 1: Identification of the phytochemical composition by LC-MS/MS of the dichloromethane fraction of young unopened leaves of *Piliostigma thonningii*.

Peak	Retention time (min)	Gross formula	Molar mass (g/mol)	MS(m/z) Ion $[M+H]^+$	Diff (ppm)	Score	Molecule name
1	21,24	$C_{12}H_{26}$	170	171	-	-	-
2	21,82	$C_{12}H_{22}O_6$	262	263	-	-	-
3	22,41	$C_{10}H_{12}O$	148	149	-	-	-
		$C_{11}H_{16}$			-	-	-
4	23,11	*$C_{15}H_{10}O_7$	302	303	2,1	97,53	Quercetin
5	23,69	$C_{15}H_{14}O_6$	290	291	-	-	-
		$C_{19}H_{30}O_2$			-	-	-
6	24,16	$C_6H_{12}O_6$	180	181	-	-	-

7	24,86	$C_{20}H_{37}O_2$	366	367	-	-	-
8	25,10	$C_{20}H_{37}O_2$	366	367	-	-	-
9	26,50	$C_{10}H_{12}O$	148	149	-	-	-
		$C_{11}H_{16}$			-	-	-
10	27,67	$C_{10}H_{12}O$	148	149	-	-	-
		$C_{11}H_{16}$			-	-	-
11	28,02	$C_{10}H_{12}O$	148	149	-	-	-
		$C_{11}H_{16}$			-	-	-
12	29,66	$C_{21}H_{36}O_3$	336	337	-	-	-
13	30,72	$C_{17}H_{32}O_4$	300	301	-	-	-
14	31,42	$C_{17}H_{32}O_4$	300	301	-	-	-
15	32,01	*$C_{20}H_{32}O_3$	320	321	1,51	97,74	En-16-α-hydroxykauran-19-oic acid
16	32,59	$C_{17}H_{32}O_4$	300	301	-	-	-
17	33,29	$C_{20}H_{30}O_2$	302	303	-	-	-
18	33,53	*$C_{20}H_{30}O_2$	302	303	1,78	60,41	Trans-communic acid
19	33,64	*$C_{20}H_{30}O_2$	302	303	1,26	96,68	Kaur-16-en-19-oic acid
20	34,11	$C_{20}H_{32}O_2$	304	305	2,38	83,37	-
21	34,34	$C_{20}H_{32}O_2$	304	305	4,51	89,93	-
22	34,93	$C_{20}H_{30}O_2$	302	303	0,88	84,76	-
23	35,40	*$C_{20}H_{32}O_2$	304	305	0,17	98,87	Anticopallic acid
24	35,90	*$C_{18}H_{35}NO$	281	282	1,98	96,03	Oleic acid amide

*: compounds identified in the young leaves of *Piliostigma thonningii*

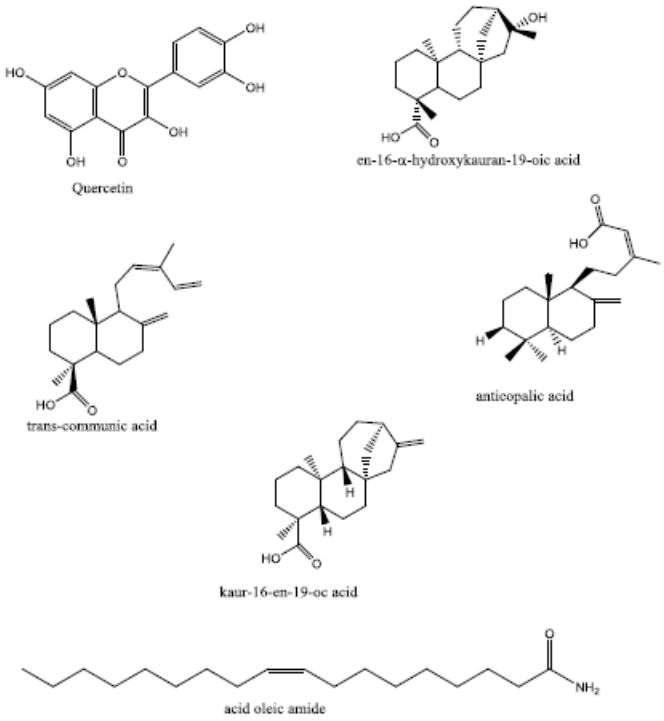

Fig. 18: Structure of compounds identified in the dichloromethane fraction of young unopened leaves of *Piliostigma thonningii*

6. CONCLUSION

Our study revealed that the young, unopened leaves of *Piliostigma thonningii* contain numerous phytocompounds. Using LC-MS analysis, we were able to identify twenty-four (24) compounds in the fraction obtained with dichloromethane. Very few of these phytocompounds remain unidentified. The unknown compounds are characterised by a high chromatographic intensity at UV wavelengths of 254 and 280 nm. These phytocompounds could therefore be the source of the plant's therapeutic properties. This highlights the use of young, unopened leaves of *Piliostigma thonningii* to treat certain diseases. This fraction could also be used to isolate unidentified molecules.

REFERENCES

1. Afolayan, M., Srivedavyasasri, R., Asekun, O.T., Familoni, O.B., Orishadipe, A., Zulfiqar, F., Ibrahim, M.A., Ross, S.A., Phytochemical study of *Piliostigma thonningii*, a medicinal plant grown in Nigeria. Med. Chem. Res, 2018; 27: 2325-2330. https://doi.org/10.1007/s00044-018-2238-1
2. Agban, A., Gbogbo, K.A., Hoekou, Y.P., Atchou, K., Tchacondo, T., Batawila, K., de SOUZA, C., Gbeassor, M., Évaluation de l'activité antifongique des extraits de Cassia alata L. et de *Piliostigma thonningii* (Schumach.) Milne Redh.(Fabaceae) sur Candida albicans. Int. J. Biol. Chem. Sci, 2013; 7: 1041-1047. https://doi.org/10.4314 / ijbcs. v7i3.12
3. Dieng, S.I.M., Fall, A.D., Diatta-Badji, K., Sarr, A., Sene, Madieye, Sene, Moussa, Mbaye, A., Diatta, W., Bassene, E., Evaluation de l'activité antioxydante des extraits hydro-ethanoliques des feuilles et écorces de *Piliostigma thonningii* Schumach. Int. J. Biol. Chem. Sci, 2017; 11: 768-776. https://doi.org/10.4314/ijbcs.v11i2.19
4. Igwe, O.U., Nwamezie, F., Comparative Studies of Phytochemicals from the Leaf and Flower Extracts of *Piliostigma thonningii* and their Antibacterial Efficacy. Int. J. Biol. Sci. Technol, 2016; 8: 11-15.
5. Kassi, A.B., Soro, Y., Fante, B., Golly, K.J., Sorho, S., Toure, A.S., Coustard, J.-M., Isolation and identification of bioactive compounds from kernel seed cake of the mango (*Mangifera indica Lam*). Int. J. Biol. Chem. Sci, 2014; 8: 1885-1895. https://doi.org/10.4314 / ijbcs. v8i4.48
6. Keïta, J.N., Koné, D., Tounkara, H., Doucouré, A., Garcia, J., Isolation and identification of three mono-glycosylated flavonoids from flowers of *Vernonia galamensis Ssp*. galamensis Var. galamensis M. Gilbert. (Isolement et identification de trois flavonols monoglycosylés des fleurs de Vernonia galamensis Ssp. galamensis Var. galamensis M. Gilbert). Maghrebian J. Pure Appl. Sci, 2016; 2: 39-46.
7. Kwaji, A., Bassi, P.U., Aoill, M., Nneji, C., Ademowo, G., Preliminary studies on *Piliostigma thonningii* Schum leaf extract: Phytochemical screening and in vitro antimalarial activity. Afr. J. Microbiol. Res, 2010; 4: 735-739.
8. Madara, A., Ajayi, J., Salawu, O., Tijani, A., Anti-malarial activity of ethanolic leaf extract of *Piliostigma thonningii* Schum.(Caesalpiniacea) in mice infected with Plasmodium berghei berghei. Afr. J. Biotechnol, 2010; 9: 3475-3480. https://doi.org/10.4314 / njpar.v31i2.69476
9. Martin, M.-T., Païs, M., Ogundaini, A.O., Ibewuilke, J.C., Ogungbamila, F.O., Complete 1H and 13C NMR Assignment of a Kaurane Dieterpene from *Piliostigma thonningii*. Magn. Reson. Chem, 1997; 35: 896-898. https://doi.org/10.1002/(SICI)1097-458X(199712)35:12<896::AID-OMR150>3.0.CO;2-S
10. Noguera, J.R., Sánchez, I.M., Carbó, M.T.D., Cortina, L.O., Miras, M.M.L., Galiano, F.B., Analytical characterisation of the biodeterioration of diterpenoid labdanic varnishes used in pictorial techniques: Sandarac and Manila copal. Int. Biodeterior. Biodegrad, 2014; 90: 99-105. http://dx.doi.org/10.1016/j.ibiod.2014.01.017
11. Pousset, J.L., Place des médicaments traditionnels en afrique. Med Trop, 2006 ; 66 : 606-609.
12. Qu, J., Wang, Y.-H., Li, J.-B., Yu, S.-S., Li, Y., Liu, Y.-B., Rapid structural determination of new trace cassaine-type diterpenoid amides in fractions from Erythrophleum fordii by liquid chromatography-diode-array detection/electrospray ionization tandem mass spectrometry and liquid chromatography/nuclear magnetic resonance. Rapid Commun. Mass Spectrom. Int. J. Devoted Rapid Dissem. Up-to-Minute Res. Mass Spectrom, 2007; 21: 2109-2119.
13. Saepou, S., Pohmakotr, M., Reutrakul, V., Yoosook, C., Anti-HIV‐1 Diterpenoids from Leaves and Twigs of *Polyalthia sclerophylla*. Planta Med, 2010; 76(7): 721-725.
14. Salawu, S., Tijani, A., Obidike, I., Chindo, B., Evaluation of anti-diarrhoeal properties of methanolic Root extract of *Piliostigma reticulatum* in rats. J. Phytomedicine Ther, 2007; 12: 4450. https://doi.org/10.4314 / jopat.v12i1.41366
15. Ukwuani, A., Ihebunna, O., Samuel, R., Peni, I., Acute oral toxicity and antiulcer activity of *Piliostigma thonningii* leaf fraction in albino rats. Bull Env Pharmacol Life Sci, 2012; 2: 41- 45.