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Comparative Chromatographic Analysis of Ethanolic Extracts of Leaves and Stem-Bark of *Piptadeniastrum africanum* (Hook.f.) Brenan and *Cathormion altissimum* (Hook.f.) Hutch. & Dandy

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ABSTRACT: This study investigated the flavonoid compounds present in the ethanolic extracts of leaves and stem-bark of *Pipitadeniastrum africanum* and *Cathormion altissimum* using Chromatographic technique with a view to assessing their degree of relatedness. The upper phase of Butanol Acetic Acid Water (BAW) solvent in the ratio of 4:1:5 was used. Results showed that the plants have several flavonoid compounds in common such as Chalcone, Flavone, Flavonol, Anthocyanin, Aurone and Isoflavonol. It was however discovered that Flavanone was present only in the leaves and stem-bark of *P. africanum* but was absent in *C. altissimum*. This discovery, which has never been previously demonstrated, may be taxonomically significant and may be justifying the placement of these plants in different genera; and by implication the monotypic status of genus *Piptadeniastrum* Brenan. There is need for further studies to determine the specific flavonoid compounds in these plants and especially the flavanone compounds that seem to be one of the chemical bases for the delimitation of the genera to which these plants belong.

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P. africanum is an important medicinal forest tree endemic to tropical Africa. This tree which also occurs in the Niger Delta especially on river banks in the riverine areas of the rain forest is validly published as the only species in the genus *Piptadeniastrum* (Brenan, 1955; Hutchinson & Dalziel, 1958; Willis, 1985; Nyananyo, 2006). It is about 45-50 m high and 3-5 m in girth with conspicuous plank-like buttresses, straight bole, with fine fern-like foliage. It has copious flowers in panicles of spikes at the end of the shoots with conspicuous, lanceolate fruits ((Hutchinson & Dalziel, 1958; Bukhill, 1995; Nyananyo, 2006). It is however considered by some indigenous people in Bayelsa State as very closely related to *C. altissimum* which is also a forest shrub or tee of about 50 ft in family Fabaceae. It is the only species of the genus *Cathormion* represented in Nigeria (Keay, 1958). The plant occurs in all of tropical Africa including Nigeria, where it is commonly found in fresh water swamp forests and secondary forests. The leaves of the plant are alternate and bipinnately compound with 4-8 pairs of pinnae. Flowers are bisexual and the fruits are often spirally twisted or curved (Lemmens, 2006). The identification and classification of plants based on chemical characters is well known, especially in the establishment of consanguinity (Davies & Heywood, 1973; Stace, 1980; Wink & Waterman, 1999). It has been acknowledged to be better than traditional morphological and anatomical methods due to the ease and flexibility of the methodology (Erdtman, 1963; Datta, 1988; Singh, 2016). One group of phytochemicals that is useful in this direction is the secondary metabolites such as flavonoids, alkaloids, terpenes, glycosides and saponins which play essential roles that enable plants to survive in their environments and reproduce successfully (Wink, 2003; Singh, 2016). The taxonomic value of phytochemicals stems partly from the fact that their

composition is very distinctive and varies widely amongst plants (Dias, 2012; Carlos & Imai, 2017). Flavonoids form one of the largest and most widespread groups of secondary metabolites and are of taxonomic significance (Wink & Waterman, 1999). The presence or absence of flavonoids compounds, for example, brought about the removal of Caryophyllaceae and Molluginaceae from ten other families of the order Centrospermae to a different order which does not correspond to existing anatomical evidence (Erdtman, 1963). Most works on *P. africanum* have concentrated on the phytochemical analysis of its leaves and stem-bark extracts with a view to assessing their antimicrobial, anti-candidosic, anti-arthritic, analgesic and anti-inflammatory properties (Brusotti *et al*., 2013; Dawe *et al*, 2016; Jiofack, 2008) with no known studies on its taxonomy in the public domain. The importance and effectiveness of paper chromatographic method in systematics is well established (Alston & Turner, 1959).This work is therefore aimed at assessing the degree of relationship between *P. africanum* and *C. altissimum* based on their phytochemical composition using the tool of paper chromatography.

MATERIALS AND METHODS

Study Area: The study was carried out in Bayelsa State which is made up of eight Local Government Areas. The state is located within latitudes $04^{0}15^{'}$ North and $05^{0}23^{'}$ south and longitudes $05^{0}22^{'}$ West and $06^{0}45^{'}$ East. It is bounded by Delta State on the North, Rivers State on the East and on the west and south by the Atlantic Ocean (Figure 1).

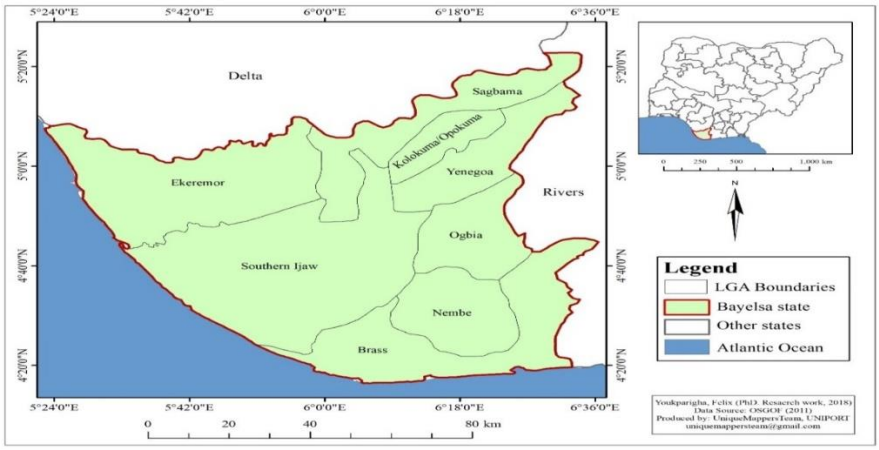

Figure 1: Bayelsa State, showing the Local Government Areas (Inset: Map of Nigeria showing Study Area).

The state has both tropical monsoon and tropical rainforest climates characterized with high humidity, dense vegetation and abundant precipitation. The wet season is not less than 340 days. The mean monthly temperature ranges from 25 ^{0}C - 31 ^{0}C. The vegetation of the State is composed of four ecological zones - coastal barrier Island forests, mangrove forests, fresh water swamps and lowland rain forests. Ecological issues facing the study area (Bayelsa State) include soil and coastal erosion, oil pollution and flooding (Johnson, 2017). Some of the common tree species are *Raphia* spp, *Cleistopholis patens*, *Alstonia boonei*, *Elais guineensis*, *Irvingia gabonensis*, *Anthocleista* spp, *Vitex grandifolia*, *Cathormion altissimum*, *Treculia africana*, *Uapaca* spp, *Symphonia globulifera*, *Taminalia* spp, *Harungana madagascariensis*, and *Alchornea cordifolia* (Ihinmikaiye & Unanaonwi, 2018). PH of surface soils in parts of the state has been reported to be generally acidic with moderate 4.7 to high 6.4. (Ezekiel *et al*., 2017) There are several surface waters such as creeks, creeklets, streams and rivers. Most of these surface water bodies have their origin from the Nun River which is a distributary of river Niger (Kigigha *et al.*, 2017).

Collection and Identification of Plant Materials: Samples of *P. africanum* and *C. altissimum* were collected from sites within the area of study. The plant specimens were morphologically identified in the Forest Herbarium Ibadan of the Forestry Research Institute of Nigeria (FRIN) and the Herbarium of the Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State. Voucher specimens of the plants were deposited in these herbaria for reference and further studies.

Extraction Method: A 10 g portion of the sample was extracted with 30 ml of 95% ethanol by boiling. This was decanted into a clean beaker and further vaporized on water bath to about 5-10 ml volume. The sample was picked up by capillary tubing and loaded into a

chromatographic paper which was marked out at reference points. After effective loading and drying of the spots, the paper was placed into a chromatographic tank-trough designed for descending order of separation. BAW was poured into the trough with the aid of a glass funnel. The tank was covered with a glass lid to allow the saturation of the space within with the gaseous phase of the solvent. The tank was placed on a stable surface until separation was accomplished i.e. when the solvent reached the designated distance marked out on the chromatographic paper. The paper was carefully removed at this point and allowed to dry on the same position in which it was placed inside the tank. When dried, the separation points were observed and recorded under visible light and under UV light before and after spraying with ammonium solution. The chromatograms were then compared with the standard chart available (Stewarte *et al*., 1974). Those that agreed with stipulated colours of flavonoids in the chart were identified as been present.

RESULTS AND DISCUSSION

The flavonoid compounds that were detected in the leaves and stem-bark extracts of *P. africanum* and *C. altissimum* under visible light and under ultra-violet light are presented in Table 1. Both plants contained Chalcone, Flavone, Flavonol, Anthocyanin, Aurone and Isoflavonol. Isoflavonol was detected only in the barks of the plants but was detected in the leaves of both plants under visible light and ultraviolet light after the chromatogram was sprayed with Ammonia solution (Table 2). The results, in Table 1, further showed that only the bark of *C. altissimum* lacked Anthocyanin, while Aurone was not found in the leaves of *C. altissimum* but these compounds were present in the bark and leaves of *P. africanum* respectively. These occurrences may not be of any taxonomic significance because it has been observed that different specimens of a plant may differ largely in chemical composition. This could be attributed to soil conditions, seasonal factors or infection-induced change in metabolism (Erdtman, 1963). This shows the importance of employing other sources of evidence to strengthen conclusions. Furthermore, results in Table 2 revealed a striking chemical difference between *P. africanum* and *C. altissimum* – the flavonoid compound Flavanone was detected both in the bark and leaf extracts of *P. africanum* but was not seen in *C. altissimum*. This restricted or unique occurrence of flavanone in *P. africanum* could be of taxonomic significance (Davies & Heywood, 1973; Wink & Waterman, 1999). The common presence of six flavonoid compounds may also be a chemical systematic evidence of their common ancestry; while the absence of flavanone in one of the plants i.e. *Cathormion altissimum* could be a clear evidence of the fact that the two plants do not belong to the same genus *Piptadeniastrum*.

Table 1: Results of paper chromatography of samples using BAW (4:1:5)

Sample	Flavonoids under visible light	Flavonoids under ultraviolet light
Leaf of *C. altissimum*	Chalcone Flavone Flavonol	Anthocyanin Chalcone Flavone Flavonol
Leaf of *P. africanum*	Anthocyanin Chalcone Flavone Flavonol	Aurone Flavonol Chalcone
Bark of *C. altissimum*	Aurone Flavone Flavonol Chalcone	Flavone Flavonol Isoflavonol
Bark of *P. africanum*	Anthocyanin Chalcone Flavone Flavonol Aurone	Anthocyanin Chalcone Flavonol Isoflavonol Aurone

Table 2: Results of paper chromatography of samples using BAW (4:1:5) after chromatogram was sprayed with Ammonia solution

Sample	Flavonoids under visible light	Flavonoids under ultraviolet light
Leaf of *C. altissimum*	Not detected	Chalcone, Flavone Isoflavonol
Leaf of *P. africanum*	Chalcone, Flavone Flavonol	Isoflavonol, Flavonol Flavanone, Chalcone
Bark of *C. altissimum*	Chalcone, Flavone Flavonol	Flavonol Isoflavonol
Bark of *P. africanum*	Chalcone	Flavone, Chalcone Flavonol, Flavanone Aurone

Conclusion: The comparative chromatographic analysis of ethanolic extracts of leaves and stem-bark of *P. africanum* (Hook.f.) Brenan and *C. altissimum* (Hook.f.) Hutch. & Dandy showed that the plants were largely similar in their chemical composition with respect to flavonoid compounds. However, flavanone occurred only in the leaves and stem-bark of *P. africanum*. This unique occurrence may be of taxonomic significance. Further studies to identify the specific flavanone compounds in *P. africanum* is recommended.

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