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Cytotoxic and growth inhibitory activity of aqueous extracts of root and leaf of *Rhaphiostylis beninensis* Planch ex Benth and *Pyrenacantha staudtii* Engl (Icacinaceae)

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Abstract
The aqueous extract of the root and leaf of *Rhaphiostylis beninensis* Planch ex Benth and *Pyrenacantha staudtii* Engl, both belonging to the family Icacinaceae were investigated for their claimed anti-tumour activity using simple bench top assays. The cytotoxic activity was evaluated within the concentration range of 1-20µg/ml using the tadpole mortality test, while the growth inhibitory activity was investigated within the concentration range of 1-30mg/ml using the *Sorghum bicolor* seed growth inhibitory test. At a concentration of 20µg/ml, aqueous extracts of the root and leaf of *Rhaphiostylis beninensis* was observed to effect a mortality of 100 and 80% respectively, while aqueous extract of the root and leaf of *Pyrenacantha staudtii*, at same concentration, effected mortality of 70 and 93% respectively. Nil mortality was observed in the control setup, while at other concentrations varying degrees of mortality was observed. All the extracts produced varying degrees of inhibition of growth in the emerging radicle of the seed of *Sorghum bicolor*. The results obtained suggest that these plants may probably have constituents with anti-tumour activities that merit further investigation using other test systems.

Keywords: *Rhaphiostylis beninensis; Pyrenacantha staudtii;* Icacinaceae; Cytotoxic; Growth inhibition

INTRODUCTION

The use of plants as a source of food, drink, shelter and medicine dates back to creation (Lambo, 1984). Extracts from plants and constituents isolated from them have been used to combat a number of disease conditions such as hypertension, malaria, bacterial infection and cancer. Cancer has been defined as the uncontrolled multiplication of cell in the body (Rang *et al*, 2007). One of the treatment options for cancer is chemotherapy. This involves the use of chemical compounds, either synthetic or from natural sources such as plant to treat cancer. Natural products from plants play a significant role in cancer chemotherapy. Some of the natural anti-cancer agents in use worldwide include palitaxel (Taxol®) from species of *Taxus*, campthothecin from *Campthotheca acuminata* and vinblastin and vincristine from *Cantharanthus roseus*. All of these exert their activity by various mechanisms, but basically they exhibit cytotoxicity and inhibit cell growth.

Rhaphiostylis beninensis Planch ex Benth (Icacinaceae) is a woody climber found growing in South-western Nigeria and the West African sub region (Bouquet and Debray, 1974). Traditionally, it is used in the management of rheumatism, skin diseases,

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mental disorder, convulsion and eye problem (Burkill, 1994; Odugbemi, 2008). It is also claimed to be useful as an insect repellent, as well in the management of abnormal growth in any part of the body (Ake-Assi and Adjanohoun, 1979). Pharmacological activities reported for the plant include anti-bacterial, analgesic and anti-inflammatory (Edema *et al*, 2006; Lasisi *et al.*, 2010; Ofeimun and Onwukaeme 2006). *Pyrenacantha staudtii* Engl (Icacinaceae) is a woody climber with green inflorescent flowers that is indigenous to the South of Nigeria and Western Cameroun (Burkill, 1994). Locally, it is used for the prevention of threatened abortion, management of dysmenorrhoea, hernia, insomnia, intestinal pain, diarrhoea and tumour related ailments (Gill, 1992; Awe *et al*, 1993). Biological activities reported for the plant include; anti-ulcer, anti-abortificent, hepatoprotective, antimalarial, phytotoxic, anti-bacterial, anti-diarrhoeal anthelminthic and anti-convulsant (Aguwa and Mittal, 1987; Falodun *et al.*, 2005; Anosike *et al.*, 2008; Mesia *et al.*, 2009; Falodun *et al*, 2009; Awe *et al*, 2011; Lasisi *et al.*, 2011; Adetutu *et al.*, 2012; Oladoye *et al.*, 2013). This study was aimed at evaluating the anti-tumour claims of both plants by investigating the cytotoxic and anti-proliferative effects of aqueous extract of leaves and roots of the plants against tadpoles of *Ranicep ranninus* and *Sorghum bicolor* (Guinea corn) seeds.

EXPERIMENTAL

Plant collection and preparation. The root and leaves of *R. beninensis* were collected in Obayantor Village near Benin, Edo State, while the root and leaves of *P. Staudtii* were collected in Ugbowo area of Benin City in March 2011. They were identified by Mr. Sunny Nweke, a plant curator at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, where a voucher specimen was deposited. Botanical authentication was carried out at the Forestry Research Institute of Nigeria (F.R.I.N), Ibadan where the voucher numbers FHI-100968, and FHI- 731051 respectively were issued. The leaves where shade dried for 5days after which they were oven dried at 40^{O}C for 1hr. The roots were washed free of earthy materials, cut into smaller pieces and dried under shade for 1wk and thereafter sun dried for 10days. Further drying was done in an oven maintained at 40^{O}C for 1hr. The different plant parts from the two plants were milled separately in an attrition mill to obtain a fine powder. 100g of each plant sample was extracted by decoction in 500mls of distilled water to obtain the aqueous extract. The extract was concentrated *in vacuo* in a rotary evaporator and preserved in well capped glass bottles at - 4^{O}C till needed.

Evaluation of growth-inhibitory activity. The protocol by Ayinde and Agbakwuru (2006) was adopted. *Sorghum bicolor* (guinea corn) seeds were purchased from a local market in Benin, and they were subjected to viability test by floating them in water. Only submerged seeds were used for this test and these were washed in methanol, blotted dry and allowed to dry further in air for 48hrs. Petri dishes (9cm diameter) were lined with 2mm-thick sterilised cotton wool followed by filter paper (Whatman I) and extract in concentration of 1, 2, 5, 10, 20 and 30mg/ml was added to each Petri dish. Seeds of *sorghum bicolor* (20) were spread out on each plate which was left half open and incubated in the dark.

The control was also set up with distilled water in place of extract. The length of the emerging radicle from the seed was measured at 24, 48, 72 and 96hrs following set up. Each determination was carried out in triplicate. Percentage growth inhibition was obtained from the formula:

$$\% \text{ growth inhibition} = \frac{A - B}{A} \times 100$$

Where A = Average length of radicle in control seeds
B = Average length of radicle in treated seeds

Evaluation of cytotoxic activity. The method of Obutor and Onojobor (2000) with a slight modification was adopted. Tadpoles were harvested from toad colonies in small water settlement within the University of Benin, Ugbowo environs. They were identified as the tadpole of *Ranicep ranninus* by Prof. M.S.I. Aisien, an animal parasitologist in the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin. Tadpoles 5-6 days old were selected and used for this evaluation. Ten viable tadpoles were selected with the aid of a broken Pasteur pipette into 250ml beakers containing 30mls of water from tadpole source, 19mls of distilled water and 1ml of extract in concentration of 0.5, 1, 2, 5, and 10mg/ml to obtain final concentrations of 10, 20, 40, 100 and 200µg/ml respectively. This procedure was carried out for the different extracts and all determinations were done in triplicates. Controls for each experiment were set up with distilled water without the extracts. The tadpoles were observed for 24hours and mortality rates were noted.

Phytochemical screening. The leaf and root of *P. Staudtii* and *R. Beninensis* respectively were subjected to phytochemical screening using standard procedures (Harborne, 1984; Evans, 2002).

Statistical analysis. Results are presented as mean ± standard error of mean (SEM) and they were analysed by one way analysis of variance (ANOVA) followed by Duncan's multiple range test. $P < 0.05$ was considered significant.

RESULTS

It was observed that the root of *R. beninensis* (RBR) gave the highest yield of 24.41% followed by the leaf of *R. beninensis* (RBR) with a yield of 18.38%. The leaf of *P. Staudtii* (PSL) (16.75%) and the root of *P. Staudtii* (PSR) gave yields of 16.75% and 12.40% respectively.

The mortality of the tadpoles which was indicated by their submergence in water and cessation of all movement was taken as an indication of cytotoxic activity. All the extracts exhibited cytotoxic effect in a concentration dependent manner. At the highest concentration of 20µg/ml, RBR extract was observed to effect the highest mortality of 100.00% with an LC_{50} > 10µg/ml. PSL extract at the same concentration (20µg/ml) produced a mortality of 93.30% with an LC_{50} of 20µm/ml. RBL and PSR extracts effected mortality rates of 80.00% and 70.60% respectively at the same concentration. The LC_{50} of the last two extracts were calculated to be 40µg/ml and 92µg/ml respectively. No mortality of tadpoles was observed in the control set up. At lower concentrations of the extracts, varying degrees of mortality was observed (Table 2).

Generally the growth inhibitory effect was observed to be concentration and time dependent. RBR at the lowest concentration of 1mg/ml effected a radicle growth of 10.28±0.30mm after 24hrs, while at the highest concentration of 30mg/ml, a radicle growth length of 1.00±0.20mm was observed, indicating a percentage inhibition of 39.00 and 93.75 respectively. At concentration of 5, 20 and 30 mg/ml after 48hrs, the growth inhibition produced by RBR was observed to be statistically different ($P<0.05$) from control. RBL at concentrations of 1mg/ml and 30mg/ml, effected radicle growth lengths of 15.53±0.30mm and 4.11±0.20mm representing percentage inhibition of 16.86 and 78.00 respectively. PSR at the highest and lowest concentrations tested (1mg/ml and 30mg/ml) produced radicle growth length of 15.15±0.20mm and 3.32±0.30mm translating to percentage inhibition of 49.65% and 95.64% respectively. The inhibitions produced by 1 and 2mg/ml of the extract after 96hrs were statistically significant ($P<0.05$) compared to control. Radicle growth lengths

of 9.33±0.20 and 1.00±0.20 were produced by 1mg/ml and 30mg/ml of PSL in guinea corn after 24 hrs of exposure representing percentage growth inhibition of 49.65 and 95.64 respectively. Growth inhibitions effected by 5, 10, 20 and 30mg/ml of the extract after 48hrs and 2, 5, 10, 20 and 30mg/ml after 96hrs were statistically different from control (P,<0.05), as the control seed were found to exhibit uncontrollable growth.

Table 1: Result of phytochemical tests

	Relative Abundance			
Constituents	PSR	PSL	RBR	RBL
Alkaloids	-	+++	+++	+
Carbohydrate	++++	++++	++++	+++
Cardiac glycoside	++	+++	+++	++
Flavonoids	+++	+++	++++	++
Reducing sugar	++	-	+++	++
Spooning				
Tannin	++	++	+++	+
Torpedoed	-	-	++	+

++++ = Abundantly present +++ = Present in high concentration
++ = Present in moderate concentration + = Present in small concentration - = Absent

Table 2: Cytotoxic effect of extracts on tadpoles

Extract	Control	1µg/ml	2µg/ml	5µg/ml	10µg/ml	20µg/ml
PSR	0.00±0.00	23.3±0.33	30.0±0.58	40.0±0.00	56.7±0.33	70.0±0.00
PSL	0.00±0.00	43.3±0.33	50.0±0.00*	66.7±0.33	86. ±0.33*	93.3±0.33
RBR	0.00±0.00	60.0±0.58	70.0±0.58	83.3±0.00*	93.3±0.33	100.0±0.00*
RBL	0.00±0.00	33.3±0.33	40.0±0.00	50.0±0.58	66.7±0.33	80.0±0.00*

Each value is mean ± standard error of mean S.E.M (n=20) of mortality. *P value < 0.05 is considered significant.

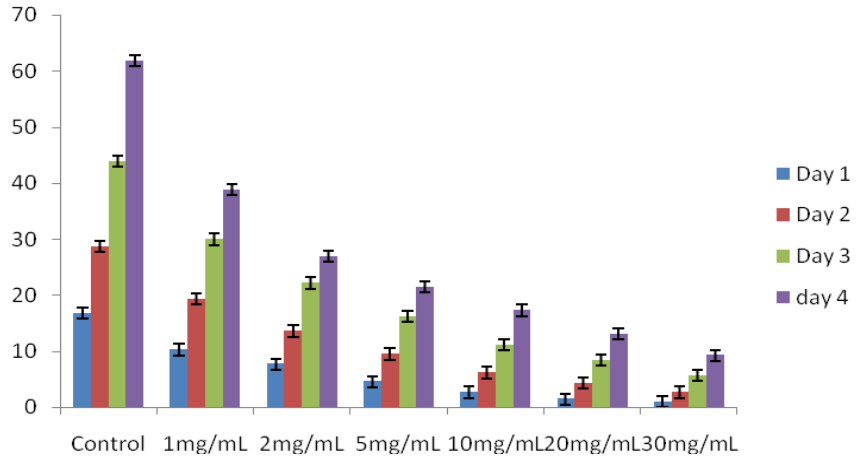

Fig 1: Growth inhibitory effect of aqueous extract of root of *Rhaphiostylis beninensis*.

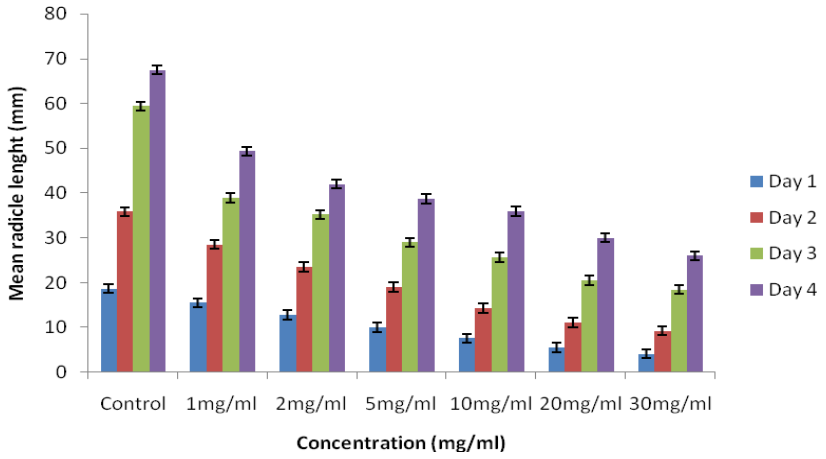

Fig 2: Growth inhibitory effect of aqueous extract of leaf of *Rhaphiostylis beninensis*.

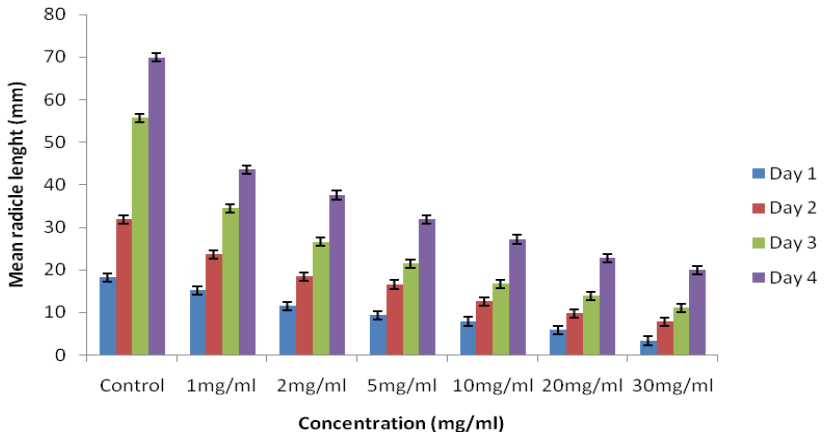

Fig 3: Growth inhibitory effect of aqueous extract of root of *Pyrenacantha staudtii.*

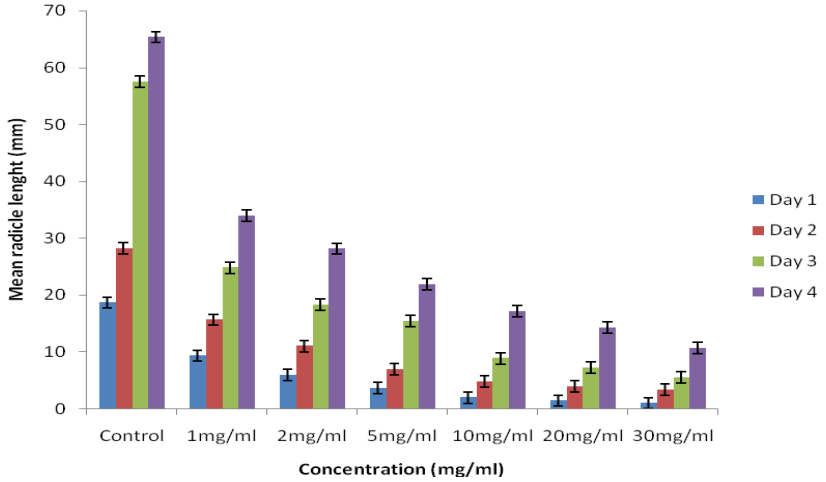

Fig 4: Growth inhibitory effect of aqueous extract of leaf of *Pyrenecantha staudtii*.

DISCUSSION

The use of simple bench top bioassay in the evaluation of the bioactivity of plant extracts especially, anti-tumour activity has long been advocated, encouraged and been in use. To serve this purpose, it is required that these bioassays be inexpensive, simple, yield quick and reproducible results (McLaughlin and Roger, 1998). The tadpole mortality and *sorghum bicolor* growth inhibition tests meet these criteria. Tumour cells are known to be highly proliferative and invasive, undergoing considerable changes often within a short time period. Tadpoles have been described as the equivalent of the embryonic stages in higher vertebrates and are thought to display considerable physiological, anatomical and histological changes during development, within a short period of time (Heywood *et al*, 2009). They are therefore thought to provide ample opportunity for interference from biological or chemical agents hence, are considered very valuable as biological indicators of toxicity. The small surface area they display as well as the easy permeability of their skin add to their value in this regard (Cooke, 1981).

Generally, seeds are known to have meristematic cells with a tendency for rapid germination and growth under favourable conditions, thus allowing obvious growth which is measurable within 24hrs. All the extracts can be inferred to have cytotoxic and growth inhibitory activities by reason of results obtained from the test systems. In control experiments in the tadpole mortality test, no death of tadpoles was observed, compared to treated tadpoles which showed varying degree of mortality. A consideration of the LC_{50} obtained for the various extract show cytotoxic activity to increase in the order; RBR→ PSL→ PSR→ RBL, with RBR having the highest activity and RBR having the least activity. In the growth inhibitory test using *S. bicolour*, seed, there was uncontrolled growth of the radicle in seeds not treated (control) with extract compared to the treated seed, where varying degree of inhibition of growth of the emerging radical was observed. The order of growth inhibitory activity inferred from the results for the different extracts is PSL→RBR→PSR→RBL

Phytochemical evaluation of the root and leaves of the plants under consideration revealed the presence of various components (Table 1). It is possible that the constituents of the extracts of the plants under consideration interfered one way or the other with the growth process of the seed of *S. bicolor* biochemically to produce the observed inhibition in the growth of the emerging radicle in the treated seeds, as well as effect mortality in the tadpoles treated with the extracts. The result obtained with the leaf and root of *R. beninensis* is in agreement with earlier studies by Lasisi *et al* (2011) in which the cytotoxic potential of the methanol extract and ethyl acetate fraction of the stem bark of the plant against brine shrimp was established. Similarly the result obtained with *P. Staudtii* leaf and root is in tandem with that obtained by Falodun *et al*, (2009) where the phytotoxic activity of the methanol extract of the leaf of the plant using the buckweed growth inhibitory test was reported. Both plants belong to the family Icacinaceae which has yielded known anti-cancer agents such as campthothecin and O-acetylcampthothecin (*Nothapodytes foetida*).

Conclusion. This study further provide scientific validation for the use of these plants as anti-tumour agents in folkloric medicine and merit further investigation involving other test systems and possible isolation of components responsible for this activity.

REFERENCES

Akubue, P.I., Mittal, G.C. and Aguwa, C.N. (1983); Preliminary pharmacological study of some Nigerian medicinal plants. *J. Ethnopharmacol* 8: 53 – 63.

Adetutu, A., Ogundare, A.O., Odeleye, A.O., Owolabi, O.Q. and Adesokan, A.A. (2012); Antibacterial and

toxicological effect of methanol leaf extract of *Pyrenecantha Staudtii* Engl. *Can J. Pure Appl. Sci.* 6: 2073 – 75.

Aguwa, C.N and Mittal, G.C (1987); Study of anti-ulcer activity of aqueous extract of leaves of *Pyrenecantha staudtii* (Icacinaceae) using various experimental models of gastric ulcer in rats. *Eur. J. Pharm.* 74: 215 – 219.

Aguwa, C.N. and Okunji C.O. (1986); Gastrointestinal studies of *Pyrenacantha staudtii* leaf. *J. Ethnopharmacol.* 15: 44 – 55.

Ake Assi, M.L and Adjanohoun E. (1979); Contribution recentsement des plantes medicinal de Cote d' Ivoire, Abidjan, p 169.

Ake, E.O., Kolawole, S.O., Wakeel, K.O. and Abiodun, O.O. (2011); Antidiarrheal activity of *Pyrenacantha Staudtii* Engl. (Icacinaceae) aqueous leaf extract on rodents. *J. of Ethnopharmacol.* 13: 148 – 153.

Anosike, C.A., Ugwu, U.B. and Nwakanma, O. (2008); Effect of ethanol extract of *Pyrenacantha staudtii* leaves on carbon tetrachloride induced hepatotoxicity in rats. *Biokem.* 20: 12-22.

Ayinde, B.A and Agbakwuru U. (2010); Cytotoxic and growth inhibitory effects of the methanol extract *Struchtium sparganophoraktze* (Asteculaceae) leaves. *Pharmacog. Mag.* 6(24), 293 – 297.

Bouquet, A. and Debray, M. (1974); Plantes Medicinales de la Cote d I'voire. Document O.R.S.T.O.M France pp 96.

Burkill, H.M. (1974); The Useful Plants of West Tropical Africa, Vol 2. Royal Botanic Gardens, Kew, United Kingdom pp 636.

Cooke, A.S. (1981); Tadpoles as indicators of harmful pollution in the field. *Enviroment. Poll.* 25: 123-133.

Edema, M.O., Iyekowa, O., Nnadi, E.F., and Eigbadion K. (2009); Chemical Characterization and anti-microbial activity of *Rhaphiostylis beninensis* root oil. *J. Chem. Soc. Nig.* 34: 10 – 112.

Evans, W.C. (2002); Trease and Evans' textbook of Pharmcognosy 15th ed. Saunders London, 42 – 44, 221 – 224.

Falodun, A., Chaudary, A.M.A, and Choudary I.M (2009); Phytotoxic and chemical investigation of a Nigerian medicinal plant. *Res. J. Phytochem.* 3:13-17

Falodun, A., Usifoh, C.O and Nworgu Z.A.M. (2005); Phytochemical and active column fractions of *pyrenecantha staudtii* leaf extract an isolated rat uterus. *Pak. J. Pharm. Sci.* 18: 31 – 35.

Gill, L.S. (Ed) (1992); Ethnomedicinal uses of plants in Nigeria. University of Benin Press, Benin City Nigeria pp 143.

Harbone, J.B. (1973); Phytochemical methods: A guide to modern techniques of plant analysis, 2nd ed. Chapman and Hall, London pp 192.

Haywood, L.K., Alexander, G.K.J., Byrne, M.J., and Cukrowska E. (2003); *Xenopus laevis* embryos and tadpoles as models for testing for pollution by zinc; copper, lead and cadmium. *Afr. Zoo.* 39(2): 163 – 174.

Lambo, J.A. (1984); Medicinal plants in tropical West Africa. Cambridge University press, London pp 142.

Lasisi, A.A., Folarin, O.O., Dara, E.O., Akinloye, O.A., and Fisuyi, M.O. (2011); Phytochemical, antibacterial and cytotoxic evaluation of *Rhaphiostylis beninensis* (Hook f ex planch) stem bark extract. *Int., J. Pharmol. Biosc.* 2(3): 489 – 495.

Lasisi, A.A., Ogunwande, I.A., Walker, M.T. and Setzer, W.M. (2006); Volatile constituents of the stem bark of *pyrenecantha staudtii* Engl. *Nat. Prod. Comm.* 2(6): 681 – 684.

McLaughlin, J.L., and Rogers L.L. (1998); The use of Biological assays to evaluate Botanicals. *Drug Interm. J.* 32: 513 – 524.

Mesia, G.K., Tona, G.L., Penge, O., Lusakiibenza, M., and Manga, T.M (2005); Antimalarial activities of three plants in Democratic Republic of Congo: *Croton mubango*, *Nauclea pobeguini* and *Pyrenacantha staudtii. Ann. Trop. Med. Parasit.* 99: 345 – 992.

Odugbemi T. Ed. (2008); A textbook of medicinal plants from Nigeria. Lagos University Press. Lagos, Nigeria pp 597.

Obuotor, E.M., and Onajobi F.D. (2000); Preliminary evaluation of cytotoxic properties of *Raphia hookeri* fruit mesocarp. *Fitoterapia.* 71: 190 – 192.

Ofeimun, J.O., and Onwukaeme, D.N. (2006); Evaluation of phytochemical constituents and pharmacological activites of the root of *Rhaphiostylis beninensis* (Icacinaceae). *J. Pharm. Sci. Phar. Prac.* (8) 3: 85 - 90.

Rang, H.P., Dale, M.M., Ritter, J.M. and Flower, R.J. (2007); Rang and Dale's Pharmacology 6th ed. Churhill Livingstone London pp 597.