Pyrenacantha staudtii Hutch and Dalz (Icacinaceae): A review of Phytochemistry, Pharmacology and Future Perspectives

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ABSTRACT

Various parts (stem bark, leaves and roots) of Pyrenacantha staudtii Hutch and Dalz, Icacinaceae are used traditionally in Nigeria and other West and Central African folk medicine in the treatment of various ailments. Databases of Scifinder, Pubmed, Google Scholar, and Medline were explored for original studies using the following search terms and the combinations thereof: Pyrenacantha, Pyrenacantha staudtii, phytochemical screening, isolated compounds, pharmacological activities, toxicity and ethnomedical uses. Search results were collated and studied, extracting relevant results to the aim of this review. The search involves all publications available (originally or translated) in English language till date.

The study revealed that different parts of P. staudtii have been used in Nigeria ethnomedicine, the Cameroon and other African countries in the management of disease conditions such as malaria, cancer, intestinal colic, threatened abortion, gout, inflammations, convulsions, menstrual disorders, ulcers and sleep disorders, in the form of decoctions, pastes and concoctions. These ethnomedical uses have also been scientifically validated by various researchers.

The results of this study do not only provide a concise overview on ethnomedical uses and pharmacology (anticancer, tocotylic, antiulcerogenic, antimalarial, insecticidal etc.) of various parts of P. staudtii but also the current stage of research on this rarely mentioned plant. It also set forth future perspectives, providing grounds for further scientific exploration of its bioactive constituents with a view of identifying drug leads and or new chemical entities.

Introduction

Pyrenacantha staudtii Hutch and Dalz belongs to the Icacinaceae family of pantropical trees, shrubs and woody climbers [1]. The genus Pyrenacantha consists of about 30 different species [2]. P. staudtii is an arborescent liane that grows up to 6 m in height and 5–10 cm in diameter; with seeds appearing as elongated and cylindrical tubercles [1], commonly distributed in the secondary jungle in Southern Nigeria, Western Cameroons, across central Africa to Uganda and Angola [2,3]. Common names of the plant in various tribes in Nigeria include Ohogha (Edo), Nhia (Ibo) and Ahara (Yoruba) [4]. Pyrenacantha staudtii is used traditionally in Nigeria and other West and Central African countries folk medicine (Plate 1).

Various parts of the plant; the leaves, stem bark and roots are used by herbalists for the treatment of different diseases and ailments: blennorhoea, hernia, insomnia, intestinal pain, diarrhea, ulcer, cancer, inflammations, dropsy, gout, threatened abortion, malaria and as a smooth muscle relaxant [1-13].

The extract of the leaf of this plant has been reported for its anti-inflammatory and antiulcerogenic activities [6,14]; smooth muscle relaxant activity [14,15]; antitumor activity [16,17]; hepatoprotective and antimalarial activities [13,18,19]; anticonvulsant, hypnotic, antiadipar and analgesic activities [20-22]. Falodun et al., (2009) reported the insecticidal activities of the oil from the leaves of this plant [23] and the phytotoxicity against Lemma minor [1].

In the Congo, the leaves are boiled in palm-wine and the infusion is taken for the treatment of blennorhoea [3]. The plant extracts usefulness in the abolition of painful uterine contractions, intestinal colic and dysmenorrheal have also been reported [6,13].

The extracts from different parts of the plant have been found to exhibit a broad range of pharmacological activities which lends credence to its ethnomedical uses. The leaf extract of the plant has been reported for its anti-inflammatory and antiulcerogenic activities [6,14].

Very few but interesting compounds have been isolated from the leaves of P. staudtii such as 3-carbomethoxy pyridine [8], bis (8 - methylhydron) phthalate and bis (8-hydroxyl-2-methylhydron) [22], amyrin and oleanolic acid [7]. The pharmacological potentials of these compounds have only partially been investigated. There is therefore the need for more research into isolation and characterization of bioactive principles responsible for the variously observed and reported activities of this plant, to completely explore their pharmacologic and therapeutic potentials. Cancer, malaria, inflammatory diseases, diabetes, gastrointestinal disorders and threatened abortion are still major public health problems that plagues majority of the...
population leading to hospital admissions, manpower and productivity loss and loss of revenue. Thus, this review is aimed at giving a critical appraisal, based on available literature evidence, on the phytochemical, pharmacological and toxicological studies of the plant and to create a platform for more research in order to discover agents, drug leads and new chemical entities that could be used against these diseases.

**Methodology**

The paper provides a concise review on the available knowledge on this rarely mentioned plant with the intent to incite research interests and explore the pharmacological activities with the aim of developing drug leads and equip the physicians’ armamentarium in the management of related ailments (threatened abortion, ulcers, asthma, diabetes, convulsions, stomach pains etc.). A systematic search through the databases of Scifinder, Pubmed, Google Scholar, and Medline for original researches using such search terms and their combinations as: *Pyrenacantha, staudtii*, isolated compounds, pharmacological activities, toxicity and medicinal uses was conducted for this review. Search results were collated and studied extracting results relevant to the review title. In doing this, searches were not limited to time or place of research but to publications available (originally or translated) in English language till date.

**Ethnomedicinal uses**

Preparations from the leaves of the plant have been employed as crude herbal decoctions and concoctions for various ailments and diseases in Nigeria, the Cameroons and other tropical countries. In the DRC for instance, the decoction of the leaves is used to treat fevers, abdominal pains, pneumonia, intestinal worms. In Nigeria decoctions of the leaf is used to treat intestinal colic, gastrointestinal bleeding; menstrual disorders such as dysmenorrhea; threatened abortion (anti-abortifient) and cancers. It is also used for the treatment of malaria and for sleep disorders.

**Phytochemistry**

The phytochemical screening of *P. staudtii* has revealed the presences of alkaloids, tannins, triterpenoidal saponins, glycosides, carbohydrates, reducing sugars, flavonoids and resins [19-24]. Phytochemical investigation has led to the isolation of some pyridine derivatives, volatile oils and related compounds from the leaves. Some compounds isolated from the leaves of *P. staudtii* include kaempferol 3-O-β-D-glucopyranosyl (1→6)-β-D-glucopyranoside (1) and 4-β-D-glucopyranosyl-(2-furyl)-5-methy-1,2-glucopyranoside phenylmethanone (2) both of which were new compounds isolated from the Icacinaceae family [25]. Other known compounds including pyridine derivatives - 3-pyridinethiocarbonylic acid (3) [25], 3-carboxethoxyphynidine (4), 3-carbobutyloxypyridine (5) [26] and 3-carboxethoxy pyridine (6) [27], sterols - β-sitosterol (7) and β-sitosterol 3-0-glucopyranoside (8); pentacyclic triterpenes - taxerol (9) [28], β-amyrin (10) and oleanolic acid (11) [29]. In addition phthalate derivatives – Bis (8-hydroxyl-2-methylthiyl) phthalate (12) and bis (8-methylthiyl) phthalate (13) were also isolated from hexane fraction of the leaf extract [30].

The structures of previously isolated compounds are shown in Figure 1. Essential oils have also been reported in the leaves of *P. staudtii* through GC-MS analysis. They include: carophyllene and caraphylene oxide, phallenden, pinene, thujene, terpinene, hexadecanoic, and tetradecanoic acids [31]. Lassis et al. [32] analyzed the components of essential oil obtained by hydro-distillation of the stem and root barks which led to the identification of 67 essential oil components where hexadecanoic acid (38.7% and 63.0%) and 1, 8-cineole (11.1% and 4.6%) were the significant components [33]. The reported analysis of essential oil components is cited in table 1.

**Pharmacology**

Based on the claimed ethnomedicinal uses, researchers have investigated a number of pharmacological activities such as anticonvulsant, hypnotic, antimalarial, anti-inflammatory, anti-schistosomiasis, analgesic, anti diabetic, antimicrobial, antioxidant, antiulcer, skeletal muscle relaxant effects, antiabortifacent and antiocytotoxic effects, anticancer and cytotoxic activities, toxicological profile and insecticidal effects of crude extracts and fractions as well as isolated compounds from *P. staudtii*.

**Anticonvulsant activities**

The in vivo anticonvulsant activity of the crude leaves extract isolated compounds from the leaves of *P. staudtii* has been investigated using different experimental models including strychnine, picrotoxin and DMSO induced convulsions [20, 22] in experimental animals as earlier described [34]. The experimental animals were pretreated with 100-400mg/kg aqueous leaf extract of *P. staudtii* administered intraperitoneally (IP) thirty minutes before the administration of IP 3.0 mg/kg strychnine and same doses of picrotoxin [35]. Results of this experiment showed a 40% protection against strychnine-induced convolution but failed to confer any anticonvulsant protection in the experimental animals against picrotoxin-induced convulsions [36]. It is possible that the mechanism of induction of convulsions by the two agents varies. Strychnine has been reported to induce convolution by the modulation of the action of glycine an inhibitory neurotransmitter [29].

Strychnine-induced convolution, its time of onset, duration of the convolution and its severity, were recorded. Sixty minutes after injection of strychnine animals were sacrificed and brains were removed for histopathological examination. The nervous system was examined for evidence of convulsions in all animals. In the control group, strychnine induced convolution, its time of onset, duration of convulsion and its severity, were recorded. Sixty minutes after injection of strychnine animals were sacrificed and brains were removed for histopathological examination. The nervous system was examined for evidence of convulsions in all animals.

The anticonvulsant activities of the crude leaf extract of *P. staudtii* were studied using the same method as above but with 100-1500mg/kg aqueous leaf extract of *P. staudtii* given intraperitoneally 30 minutes before picrotoxin to show the protection against picrotoxin-induced convolution.

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Kasuret al. [20, 21], reported that a fraction of extract which contain majorly triterpenoids exhibited anticonvulsant activity against electroshock (MES), pentyleneetetrazol (PTZ), strychnine, lithium-methylnonyl) phthalate and electrical kindling. The mechanism of their actions was linked to a possible involvement of GABAergic neurons. In this study, the extract showed increased GABA content in the brain which could be the likely mechanism of anticonvulsant action of the extract, since drugs increasing GABAergic transmission inhibit seizures [34-36]. The results were however not the same with compound 2. All the animals treated with compound 2 died just after the convolution even though there were marginal increases in extension of the whole body and mortality. In the experimental groups, the animals were pretreated with different concentrations of isolated compounds bis(8-hydroxyl-2-methylthiyl) phthalate (1) and bis (8-methylthiyl) phthalate (2) after which they were given 2% DMSO (10 mg/kg) by IP route and observed for tonic convulsions and lethality over a 24-h period. The results of this study showed there was a dose-dependent increase in the latency minutes of tonic convulsion from 5 to 20 minutes with doses of 1 mg/kg and 4 mg/kg of compound 1, respectively with synonymous decrease in percentage mortality 80% compared to the control group. There was also an increase in latency of death minutes compared to control.

Awe et al. [37] reported the hypnotic effect of *P. staudtii* extract using hexobarbitone -induced sleep in mice model described by Leite et al. [38], 85.0 mg/kg hexobarbitone was injected into the mice (mice) IP.
followed by IP 100.0 – 400.0 mg/kg of the extract. The parameters used for assessment included loss of writhing reflex to detect the onset of hypnosis, and the return of writhing reflex as index of awakening. The results of this experiment showed potentiation of hypnosis (duration of sleep in minutes) induced by hexobarbitone (P < 0.05) compared to the control (10 mL/kg normal saline). This effect was dose-dependent, increasing from 5.4 ± 0.4, 15.6 ± 0.8 to 28.4 ± 0.2 for 100.0 mg/kg, 200.0 mg/kg and 400.0 mg/kg respectively [31].

**Antiulcerogenic activities**

The antiulcer activities of the aqueous leaves extract of *P. staudtii* against ulcers induced by different experimental models: aspirin, indomethacin, serotonin, reserpine [3], cold-restraint stress and Shay rat [33], have been studied. In one study [4], the aqueous extract of the leaves of *P. staudtii* was found to have significant antiulcer activity against all the models studied. In the other [33], the protection against ulcers was found to be dose-dependent in the case of indomethacin-induced ulcers. In this study, different fractions of the leaf extract exerted different antiulcer activity. With the ethanol extract showing the greatest ulcer protective effects (P < 0.001), only 20% of the experimental animals developed ulcers and having very low ulcer index. While in the serotonin induced ulcers, cimetidine a known antiulcer drug did not exert any significant protective effect compared to ethanol extract of the leaves of *P. staudtii* which showed significant (P < 0.05) ulcer protection in the experimental animals as measured by the ulcer index with only 20% showing evidence of ulcers [33].

In the Shay rats experiment (pyloric ligation induced ulcers), the extract and cimetidine exerted significant P<0.01 antiulcer activity when compared with the control group. In similar vein, 90% of the experimental animals had ulcers in the cold-resistant stress induced ulcers, while the ethanol extract group showed 60% protection compared to 50% of the cimetidine group. There was a dose dependent increase in the ulcer protection with *P. staudtii* extract as shown by dose dependent experiment. With an oral effective dose of 28.5 mg/kg of the extract, only 30% of the animals developed ulcers. Development of ulcers in the experimental animals was nil when the dose was doubled [5]. It was also reported in this study that the mechanism of action of the extracts may be due to a protective effect on the gastric mucosa, since the most active fraction of the plant extract was said to be rich in triterpenoidal saponins. Carbenoxolone a triterpenoidal related compound was found to be effective as an antiulcer agent [76,77,78]. This action was attributed to selective inhibition of prostaglandin PGF2α. In a recent work [34], the gastric mucosa protective effect of carbenoxolone was attributed to the inhibitory effects that it exerts on 15-PGDH which leads to increase PGs levels in the stomach. Furthermore, *P. staudtii* extract significantly reduced the incidence of reserpine induced ulcers P<0.05. The ulcer index was reduced from 0.90 ± 0.29 to 0.16 ± 0.09 and only 30% of the animals developed ulcers [35]. Whereas cimetidine did not prevent reserpine induced ulcers, instead it potentiated the ulcerogenic effect of reserpine, the ulcer index increasing from 0.90 ± 0.29 to 1.38 ± 0.45. Akubue et al. [4] reported the significant (P < 0.05) reduction of incidence of histamine-induced gastric ulcers in rats as described by [33] following the oral administration of 1 mL of aqueous extract of *P. staudtii*. Different workers [34-36] reported the gastric mucosa protective activities of different medicinal plants. These studies agree with that of Agwu and Mital [33].

**Anti-inflammatory, analgesic activity and antipyretic activity**

The *in vivo* analgesic effects of the aqueous leaf extract of *P. staudtii* was reported [29]. The acetic acid-induced abdominal constriction [36] and formalin-induced paw licking [37] methods in mice were employed. In both methods, different concentrations of the extract (100.0, 200.0 and 400.0 mg/kg) were given intraperitoneally, with indomethacin (5.0 mg/kg IP) as a reference and standard analgesic drug. The aqueous leaf extract of *P. staudtii* at the given doses (100.0 – 400.0 mg/kg, IP) exhibited significant and dose-dependent analgesic effect in the acetic-acid induced writhing test in mice (p < 0.05) compared to the control, 10 mL/kg normal saline. At 200 and 400 mg/kg, the analgesic effects were comparable to 5.0 mg/kg indomethacin with total number of writhing 2.0 ± 2.7 and 0.6 ± 0.8, respectively while indomethacin elicited 3.3 ± 0.8 writhings. Also in the paw-licking induced by formalin study, the results were interesting. There were two distinct phases in this test which reflected different types of pain [37, 38]. There was an early phase that reflected a direct effect of formalin on nociceptors (neurogenic pain) and the late phase which reflected tissue injury or inflammatory pain [37, 38]. It was observed that the extract produced significant anti-nociceptive effect in both phases. From this study, it is probable that the extract exerts its analgesic effect through two pathways: peripheral inhibitory actions due to the release of prostaglandins (inflammatory pain) and central activity due to antagonistic action of the nociceptors (neurogenic pain) [39].

**Antidiarrheal activity**

The antidiarrheal effects of *P. staudtii* was investigated in experimental animals (mice and rats) using different experimental models: castor oil-induced diarrhea in rats; castor oil-induced enteropooling, intestinal transit and intestinal fluid in rats; castor oil-induced enteropooling, intestinal transit and intestinal fluid in mice and the effects of gastrointestinal motility of *P. staudtii* extracts compared to atropine in mice [20]. In this study 3 mL/kg castor oil was administered per oral (p.o) to the animals 30 minutes after treatment of the animals with aqueous extract of *P. staudtii* at doses of 100, 200 and 400 mg/kg and 10 mg/kg loperamide a standard antidiarrheal agent. This was followed by 6-hour observation of the
animals for the presence of characteristic diarrheal droppings. The parameters observed include: the time elapsed between the administration of castor oil and the excretion of the first diarrheic feces, the total number of fecal output and the number of diarrheic feces excreted by each animal in a specific period of time. The weight of the diarrheic feces in mg at certain time. Severity of the castor-oil induced diarrhea was noted and scored using the method of Dicarlo et al. [73]. In the castor oil-induced enteropooling, intestinal transit and intestinal fluid in rats, the same procedure as above was followed except that the doses of extract and drug was followed by the administration of 3 mL/kg (p.o.) of 10% activated charcoal in physiological saline (0.9%/w/v sodium chloride solution) and the intestinal transit and volume of intestinal fluid measured until the end of experiment.

In the castor oil-induced enteropooling, enteropooling, intestinal transit and intestinal fluid in mice, same procedure was repeated as with rats. Similarly, in the gastrointestinal motility of P. staudtii against atropine in mice, same procedure as in 2 and 3 except that group 6 mice were treated with atropine sulphate (1 mg/kg, p.o.) followed thirty minutes later by 3 mL/kg of 10% activated charcoal meal p.o. The results of the studies showed that the aqueous extract of P. staudtii (100–400 mg/kg, p.o.) produced significant and dose dependent inhibition of all the diarrheal parameters (onset, frequency and severity of diarrhea, total number of stools, number of wet stools, and weight of wet stools) investigated. At these doses, the extract produced significant (P < 0.05–0.01) delayed in the onset of diarrhea when compared to the controlled group (water), however loperamide (10 mg/kg, p.o.) the standard antidiarrheal drug produced a more marked inhibitory effect (P < 0.001).

The normal intestinal propulsive movement and transit time in rodents were significantly and dose- dependently decreased by the extract comparable to those of the standard antidiarrheal drugs, loperamide (10 mg/kg, p.o.) and atropine (1 mg/kg, p.o.). Loperamide and atropine inhibit gastrointestinal motility (propulsion), reduce intestinal fluid secretion and accumulation, and delay gastric emptying, thus ameliorating diarrhea. It was also noted that charcoal meal traversed the fastest distance of the total length of the intestine in the control group compared to P. staudtii aqueous (100, 200 and 400 mg/kg, p.o.) leaf extract-treated test groups of mice. There was a dose dependent and significant (P < 0.05–01) decreased in the normal intestinal propulsive movement and transit of charcoal meal through the small intestine. However, loperamide (10 mg/kg, p.o.) produced higher antimitoty effect than the highest dose of P. staudtii (400 mg/kg, p.o.) used. It was observed that thirty minutes after intra-gastric administration in the control group of rats, the charcoal meal traversed 94.13% of the total length of the small intestine. P. staudtii extract at doses (100, 200 and 400 mg/kg, p.o.) exhibited significant (P < 0.05–0.01) decreased in the propulsive movement and transit of charcoal meal through the small intestine. Loperamide (10 mg/kg, p.o.) and atropine produced similar and greater antimitoty effect than the highest dose of P. staudtii extract (400 mg/kg, p.o.) used in rats and mice respectively.

These effects were similar in the castor oil-induced fluid accumulation. Here the effects of P. staudtii extract were significant (P < 0.05–0.01) while loperamide (10 mg/kg, p.o.), produced a significant (P < 0.001) inhibitory effects on castor oil-induced fluid accumulation. It has been reported that castor oil induces diarrhea due to its active ingredient ricinoleic acid [44] which stimulates the production of several mediator substances that include prostaglandins, nitric oxide, and platelet activating factor, cAMP and tachykinins synthesis. P. staudtii (PS) has been shown by different workers to possess smooth muscle relaxant activity and also relaxes guinea-pig isolated ileum, antagonized acetylcholine (ACh) and histamine (HT)-induced contractions [13, 15] the effect of which is thought to be due to inhibition of calcium mobilization. Reynolds et al. [45] proposed that calcium antagonism is responsible in part for the antidiarrheal effect of loperamide and atropine. P. staudtii contains an IC50 value of 60 μg/mL of calcium-antagonists like verapamil and diltiazem which exhibit spasmylocic properties on guinea pig ileum [45]. It could be inferred from the above that PS diarrhea and motility inhibitory effects could be due to the presence of phytoconstituents acting as calcium antagonist on one hand and by mediation of antimitoty effects by blockade of muscarinic cholinoreceptors and/or histaminic receptors on mammalian intestine. Therefore, PS may be exhibiting its antimitoty activity by the same pathway as atropine a muscarinic cholinoreceptor antagonists which could synergistically potentiate the antimitoty effect of P. staudtii extract by enhancing the extract’s effect on both intestinal secretion and motility.

Anticancer and Cytotoxicity activity
In order to establish the ethnomedicinal claims for the use of P. Staudtii extract in the treatment of various ailment including cancer Engel et al. [16] investigated the in vitro anticancer effects of the ethanol extracts of P. staudtii against human epithelial MCF-7 breast cancer cell lines in a dose-dependent manner (1–50 μg/mL) by using cell cycle analysis, MIT viability assay, annexin V/propidium iodide (PI) staining, TUNEL method and the determination of apoptotic and adhesion relevant proteins. The experimental cell line (MCF-7) was treated with different concentrations of the plant extract (1, 10, 25, 50 μg/mL) for 48 h in assay medium according to the method of Engel et al. [16]. DMSO (0.1%) was used as the negative control vehicle.

Results of this study showed that the extract at the given concentrations induced (concentration dependent) significant inhibition in the proliferative (S and G2/M) phases of the MCF-7 cell. The incidence of apoptosis however was measured by the determination of the subG1/peak which showed DNA fragmentation events. Extract of P. staudtii was shown to mediate a linear reduction in proliferation. The extract caused a significant increase in sub-G1 phase, an indication of arrest or blockade of the G2/M phase. This influence on proliferation rates of the estrogen receptor-positive cell line MCF-7 is a common phenomenon with some effective anticancer extracts. Genistein a natural anti-cancer agent is known to induce apoptosis in several tumor cell lines [46, 47]. Genistein in low concentrations (1–10 μM) stimulates cell proliferation but at concentrations >10 μM it induces a blockade in the G2/M phase. The result of this experiment suggests substances similar to genistein phytoestrogens may be present in this plant. Ravindranath et al. [48], reported antitumor activities of genistein a soy isoflavone that acts by inhibiting various kinases responsible for the induction of cancer cells. These kinases are involved in the expression of cytokines and stimulators of the activation of cancer. Genistein alone at 100 μg/mL of an aqueous extract of P. staudtii resulted in approximately 33% cell death compared to the control. PS does not only induce necrotic events verified by high amounts of PI-positive cells, but also cause fragmentation within the cell nucleus. P. staudtii also produce morphological alterations as a result of down regulation of β-integrins. The extract at concentration of 10 μg/mL or higher induced a loss of cell-cell and cell matrix adhesion accompanied by shrinking of the cells and detachment of the extracellular matrix from cell-cell and extracellular matrix adhesions which promotes metastasis. This detachment from the extracellular matrix or lack of contact with neighboring cells is a clear indication of induction of an apoptotic process that is termed anokia [48]. Anokia was confirmed by live/dead staining of the cells after exposure to 10 μg/mL of the extract. In this process only attached cell, i.e. viable cells pick up the green fluorescence while detached cells (dead) appear red. In comparison, with flow cytometry data, 10 μg/mL of P. staudtii caused a significant increase in β1-integrin expression in the cell membrane fraction while the soluble protein content was reduced. This phenomenon may be attributable to the fact that different antibodies were used for the flow cytometry analysis. The antibody suitable for western blotting detection of β1-integrin was raised against amino acids 375–480 mapping within an extracellular fragment of β1-integrin of human origin (sc-374429, Santa Cruz). Exposure of the cells to P. staudtii caused a slight decrease in the auto phosphorylation of FAK. FAK (focal adhesion kinase) is a cytoplasmic protein tyrosine kinase that is involved in integrin-mediated signal transduction and its phosphorylation. This decrease in the auto phosphorylation of FAK is an indication of the deactivation of FAK and thus the reduction of cells adhesion to the extracellular matrix [17, 18]. P. staudtii showed an IC50 of 10–50 μg/mL which differ remarkably from cell cycle analysis, bright field microscopy and β1-integrin expression revealed marked lower effective concentrations (1–10 μg/mL) of the plant extract [17]. The cytotoxic activity and growth inhibitory activity of the aqueous root and leaf extracts of Pyrenacantha staudtii were investigated [49] using the tadpole mortality test and the Sorghum bicolor seed growth inhibitory test, respectively. The cytotoxic activity was evaluated within the concentration range of 1–20

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Figure 1: Structures of compounds 1-13 isolated from P. staudtii
µg/mL, while the growth inhibitory activity was investigated within the concentration range of 1-30 mg/mL. Results of this study show that at the concentration of 20 µg/mL, the extracts induce mortality of 70 and 80% in the tadpole mortality test. There was no mortality in the control group. With the emergence of these oxidant dysfunctioning drugs, the xanthine oxidase inhibitory effect of emerging radicle of the seed of *Sorghum bicolor*. Shweta et al., [16] isolated camptothecine a known anticancer drug from the fruits of *Pyrenacantha volubilis* (Icacinaceae) a species of the genus Pyrenacantha and showed its anticancer activities. They also reported the effectiveness of the seed extracts of this species against breast cancer, ovarian, colon and carcinoma cell lines (with IC₅₀ values of 4.0 µg/mL, 6.5 µg/mL, 25.0 µg/mL and 25.0 µg/mL respectively). This study is in agreement with those of [14, 15], that members of the family Icacinaceae possess anticancer activities.

**Smooth muscle relaxant activity**

The smooth relaxant activity of 3-carbomethoxy pyridine isolated from *P. staudtii* [10] and its derivatives 3-carbohexoxy pyridine and 3-carbomethoxy pyridine were investigated against oxytocin induced spontaneous uterine contractions of isolated rat (Wistar rats) uterus. Salbutamol was used as the positive control drug. The results show significant inhibition of contractions by all compounds. 3-carbomethoxy pyridine produced significant (P < 0.05) uterine inhibitory effect when compared to oxytocin alone at a dose of 20 mg/mL. 0.1 IU of oxytocin induced percentage response of 37% of the maximum given alone. This was however reduced to 5% when co-administered with 3-carbomethoxy pyridine with subsequent reduction in spontaneous muscle contractions, which was restored after 4 minutes. On the other hand, 3-carbomethoxy pyridine exerted significant (P < 0.05) and marked inhibitory effect on the oxytocin induced contractions with total elimination of the spontaneous contractions of the uterus. With 3-carbohexoxy pyridine, spontaneous uterine contractions were restored 1 h after washing. This could be an advantage in threatened abortion, since this compound (3-carbomethoxy pyridine) abolishes oxytocin induced uterine smooth muscle contractions and spontaneous contractile activity. The inhibitory effect of 3-Carbobutoxy pyridine on oxytocin induced and spontaneous contractions was also significant (P < 0.05) at a dose of 20 mg/mL. This relaxation was comparable to salbutamol, a prescription drug used as a smooth muscle relaxant. The study shows that 3-Carbobutoxy pyridine unlike the other two agents completely inhibit uterine contractions without any spontaneous activity even at higher doses of oxytocin [14]. The increment in the alkyk substituent which also increases the lipophilicity of these compounds is somewhat correlated with increase in the smooth muscle relaxant effects of the compounds. Salbutamol at a dose of 30 mg/mL induces significant (73.54%) relaxation of uterine contractions compared to 20 mg/mL (100 ± 0.00) of 3-carbomethoxy pyridine [14].

**Xanthine oxidase inhibitory activity**

The Xanthine oxidase inhibitory effects of *P. staudtii* isolated compounds was evaluated by Falodun et al., [23] using Allopurinol as a standard drug and DMSO as negative control. The IC₅₀ values determined by EZ-FIT windows-based software shows that the two new compounds (kaempferol 3-O-ß-fl-hamponoprasyroyl (1→6)-ß-D-glucopyranoside and 4-ß-glucopyranosyl (2-furyl)-5-methyl-2-glucopyranoside phenyl/methanone) have 23 % and 39 % xanthine oxidase inhibitory effects compared to Allopurinol (IC₅₀ 10.23 ± 2.67). However, the known compound 3-pyridinecarboxylic acid also exhibited some xanthine oxidase inhibitory effect at 68 % (IC₅₀ 518.23 ± 2.23). Xanthine oxidase is an enzyme which is involved in defending the body against external agents during inflammatory responses. It usually produces superoxide by the reduction of molecular oxygen and oxidation of xanthine to uric acid which is a parallel event. Its activity in neutrophils during inflammation is more compared to other sites of the body. It is found to play a critical role in diabetes and other metabolic disorders, therefore the discovery of its inhibitors may lead to tremendous efforts in drug development to treat such type of diseases in which xanthine oxidase is involved. The percentage inhibitory activity by the samples was determined against a DMSO blank IC₅₀ of samples was determined by using EZ-FIT windows-based software. Its activity has been known to play an important role in the progression of vascular endothelial dysfunction (VED) and the xanthine oxidase pathway is a major pathway in the generation of vascular oxidative stress [15]. Xanthine oxidoreductase (XOR) is part of a group of enzymes known as the molybddehyde iron-sulfur flavin hydroxylases first discovered in milk [23] and believed to be involved in reactions that produce reactive oxygen species (ROS). XOR is reported to be the only enzyme capable of catalyzing the formation of urate in man. XO is significantly elevated in a variety of conditions including limb ischemia [53] major surgery [54] coronary artery disease and heart failure [55]. Allopurinol, a xanthine oxidase inhibitor, has been in clinical use for over 40 years in the treatment of chronic gout. Allopurinol has also been shown to improve hyperuricemia, reduce oxidative stress burden and improve myocardial efficiency by reducing oxygen consumption in smaller mechanistic studies involving various cohorts at risk of cardiovascular events [51].

**Antimalarial activity**

The antimalarial activity of the methanol extract and fractions of the leaves of *P. staudtii* were investigated in vitro and in vivo against *Plasmodium falciparum* and *Plasmodium berghei* in mice. In the in vitro assay, the dichloromethane fraction of *P. staudtii* with IC₅₀ < 1 µg/mL, was the most active followed by the aqueous extract IC₅₀ value of 15.2 µg/mL. The in vivo study revealed that at a daily oral dose of 200 mg/kg, the chloroformic, ethylacetate and n-butanolic fractions of *P. staudtii* showed 94.4%, 98.7%, 84.9% and 99.3% chemosuppression of parasitaemias, respectively, by day 4 in the *P. berghei berghei* infected mice in contrast to 37% chemosuppression observed with the aqueous extracts. The study however reported that the mice treated with the 80 % methanol extract of *P. staudtii* had higher parasitaemia on day 4 than the negative control mice by day 4 of the experiment [13].

**Insecticidal activity**

The insecticidal activity of the essential oils of *P. staudtii* was evaluated against * Tribolium castaneum* (TC) and *Rhizophyta dominica* (RD) two known beetles (both are pests of stored maize and a variety of stored products). Permethrin and ethanol were used as positive and negative control agents, respectively. At doses of 1.0919 mg/mL, the percentage insecticidal activity of the essential oils of *P. staudtii* against the studied insects were 60 ± 1.44 (TC) and 80± 1.22 (RD) compared to the positive control agent Permethrin which showed 100% insecticidal activity at a dose of 237.5 µg/mL [21]. Permethrin is a pyrethroid insecticide, and exerts its effects on insects’ nervous system by inhibition of sodium ion influx through nerve cell membrane channels resulting in delayed repolarization and thereby leading to paralysis and eventual death of the pest [56]. The possibility of essential oils of *P. staudtii* having similar mechanism of action could be inferred. The insecticidal activity of *P. staudtii* could be attributed to some of its essential oil components such as linalool (31.3%), limonene (19.4 ), 1,8-cineole (14.7%), phallendrene (12.45 %); hexadecanoic (17.7 %) which have been shown by different workers to have significant insecticidal activity against rice weevil *Sitophilus oryzae* and *Rhizophyta dominica* [57, 58]. Linoleic acid has also been reported to exhibit high toxicity against *instar larvae with LC₅₀ values ranging from 4.78 - 9.11 g/100 mL [59]. Hexadecanoic acid has been shown to exhibit larvicidal effect against *Plasmodium falciparum* [60]. Essential oils of *P. staudtii* could be a lead for insecticidal agent against most store insects.

**Acute and Sub-acute toxicities effects**

Anoseki et al., [66] and Mesia et al., [13] reported the acute and sub-acute toxicity effects of extracts and fractions of *P. staudtii* in experimental animals (Wistar rats and Swiss mice). In both studies the extracts/fractions did not show any external or histological toxicity effects on the animals at extract doses > 5 g/kg body weight (LD₅₀ > 5 g/kg body weight). Toxicity effects were investigated after 30 days of administration in experimental animals. *P. staudtii* seeds were similar in neutrophils during inflammation in the controls were 85.3 ± 2.4 (male) and 85.6 ± 2.7 (female) compared to the treated group 86.7 ± 4.3 and 86.7 ± 3.2 male and female mice, respectively. In another study [61] chronic oral administration of *P. staudtii* extracts did not show any significant effects on the hematological indices of the animals used in the study. White blood cells count of all the treated animals (rats) were within normal limits compared to the control (p < 0.05) throughout the treatment period. However, there were significant changes in PCV, Hemoglobin and RBC with chronic oral administration of the extracts (30 mg/kg body weight).

**Hepatoprotective effects**

Various toxic agents (antibiotics, chemicals, CC14, microbes and excessive consumption of alcohols) have been implicated in liver cell injuries. This is further complicated by the fact that available synthetic drugs used in the
The authors declare no conflict of interest.

Dietary investigations in order to tap the therapeutic potential of the extracts obtained from different parts of the plant have exhibited the presence of alkaloids, tannins, triterpenoidal, and flavonoidal compounds. The results of the study have shown that the extracts have significant antioxidant and antimicrobial activities.

Conclusion

P. staudtii a woody climber endemic to the tropical rain forest of Nigeria, the Cameroons and into Central Africa has been employed in traditional medicine practice in these regions. Different parts of the plant (leaves, roots, stem bark and tubers) have been used in various forms (decoctions, infusions, pastes and ointments) as remedics for diseases affecting man, such as cancer, malaria, inflammations, ulcers, blemorrhoea, threatened abortion, menstrual disorders, convulsions and sleep disorders. This review has endeavored to give an overview of the ethnomedicinal uses, pharmacology and phytochemistry of P. staudtii. Phytochemical screening of various parts of the plant revealed the presence of alkaloids, tannins, triterpenoidal compounds, saponins, glycosides, carbohydrates, reducing sugars, flavonoids, resins. Various fractions and extracts from different parts of the plant exhibit numerous pharmacological effects in experimental animals and in vitro studies such as antitumor, anticonvulsant, antiulcerogenic, hepatoprotective, antiinflammatory, and antiplatelet activities.

Conflict of interest

The authors declare no conflict of interest.
References
