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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 ethnomedicinal 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. staudti* have been used in Nigeria ethnomedicine, the Cameroons 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 ethnomedicinal uses have also been scientifically validated by various researchers.

The results of this study do not only provide a concise overview on ethnomedicinal uses and pharmacology (anticancer, tocolytic, antiulcerogenic, antimalaria, 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 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) [3]. *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: blennorrhea, hernia, insomnia, intestinal pain, diarrhea, ulcer, cancer, inflammations, dropsy, gout, threatened abortion, malaria and as a smooth muscle relaxant [3-13].

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In the Congo, the leaves are boiled in palm-wine and the infusion is taken for the treatment of blennorrhoea [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 ethnomedicinal uses. The leaf extract of the plant has been reported for its anti-inflammatory and antiulcerogenic activities [6,14], smooth muscle relaxant activity [14,15], anticancer activities [16,17], hepatoprotective and antimalarial activities [13.18,19], anticonvulsant, hypnotic, antidiarrheal 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 *Lemna minor* L [7].

Very few but interesting compounds have been isolated from the leaves of *P. staudtii* such as 3-carbomethoxypyridine [8], bis (8 - methylnonyl) phthalate and bis (8-hydroxyl-2-methylnonyl) [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

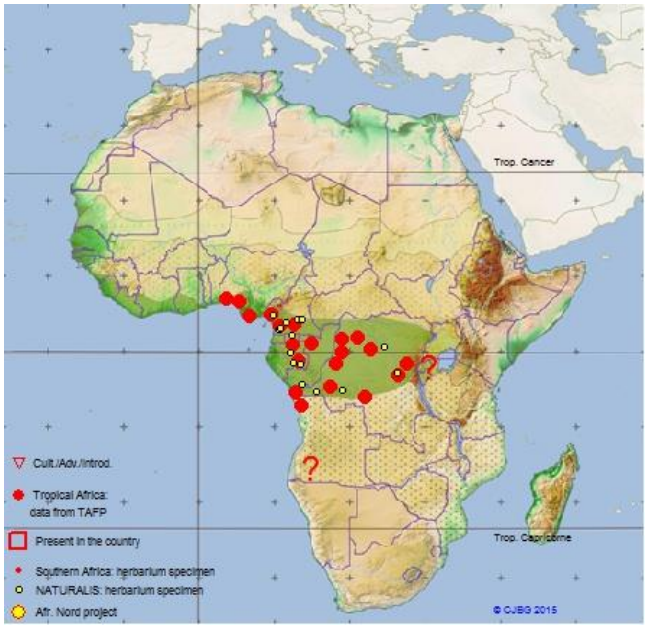

Plate 1: Geographical distribution of *P. staudtii* through Africa (Red dots). Adopted from African Plant Database.

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, diarrhea, 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 dysmenorrheal; threatened abortion (anti-abortificient) 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 kaempherol 3-O-*β*-rhamnopyranosyl (1→6)-*β*-D-glucopyranoside (**1**) and 4-*β*-glucopyranosyl-(2-furyl)-5-methy-l,2-glucopyranoside phenylmethanone (**2**) both of which were new compounds isolated from the Icacinaceae family[25]. Other known compounds including pyridine derivatives - 3-pyridinecarboxylic acid (**3**) [25], 3-carboethoxypyridine (**4**), 3-carbobutoxy pyridine (**5**) [14] and 3-carbomethoxy pyridine (**6**) [8]; sterols - *β*-sitosterol (**7**) and *β*-sitosterol 3-*O*-*β*-glucopyranoside (**8**); pentacyclic triterpenes - taraxerol (**9**) [25], β-amyrin (**10**) and oleanolic acid (**11**) [7]. In addition phthalate derivatives – Bis (8-hydroxyl-2-methylnonyl) phthalate (**12**) and bis (8-methylnonyl) phthalate (**13**) were also isolated from hexane fraction of the leaf extract [22].

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 carophylene oxide, phallenden, pinene, thujene, terpinene, hexadecanoic, and tetradecanoic acids [23]. Lasisi *et al*. [24] 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 [24]. 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, antidiabetic, antimicrobial, antioxidant, antiulcer, skeletal muscle relaxant effects, antiabortifacient and antitocolytic 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 and 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 [26]. 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 [20]. Results of this experiment showed a 40% protection against strychnine-induced convulsion but failed to confer any anticonvulsant protection in the experimental animals against picrotoxin-induced convulsions [20]. It is possible that the mechanism of induction of convulsions by the two agents varies. Strychnine has been reported to induce convulsion by the modulation of the action of glycine an inhibitory neurotransmitter [27, 28] indicating a possible involvement of glycinergic transmission, whereas convulsions induction effects of picrotoxin is due to its antagonistic effects on GABA receptors [29], an indication that the extract did not potentiate GABA transmission.

In the DMSO – induced convulsion method the control group received 2% (10 mg/kg) DMSO IP and the animals were observed for symptoms of convulsion which include: masticatory movement, head nodding and myoclonic jerking of limbs followed by wild running and subsequent tonic 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-methylnonyl) phthalate (**1**) and bis (8-methylnonyl) 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.

Kasture *et al*.[30], reported that a fraction of extract which contain majorly triterpenoids exhibited anticonvulsant activity against electroshock (MES), pentylenetetrazol (PTZ), strychnine, lithium-pilocarpine 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 [31, 32]. The results were however not the same with compound **2**. All the animals treated with compound **2** died just as in the control even though there were marginal increases in latent minute of tonic convulsion and latent death minute compared to the control. This shows that compound **2** may not be effective as an anticonvulsant drug [22].

Awe *et al*, [20] reported the hypnotic effect of *P. stuadtii* extract using hexobarbitone -induced sleep in mice model described by Leite *et al*. [79]. 85.0 mg/kg hexobarbitone was injected into the animals (mice) IP

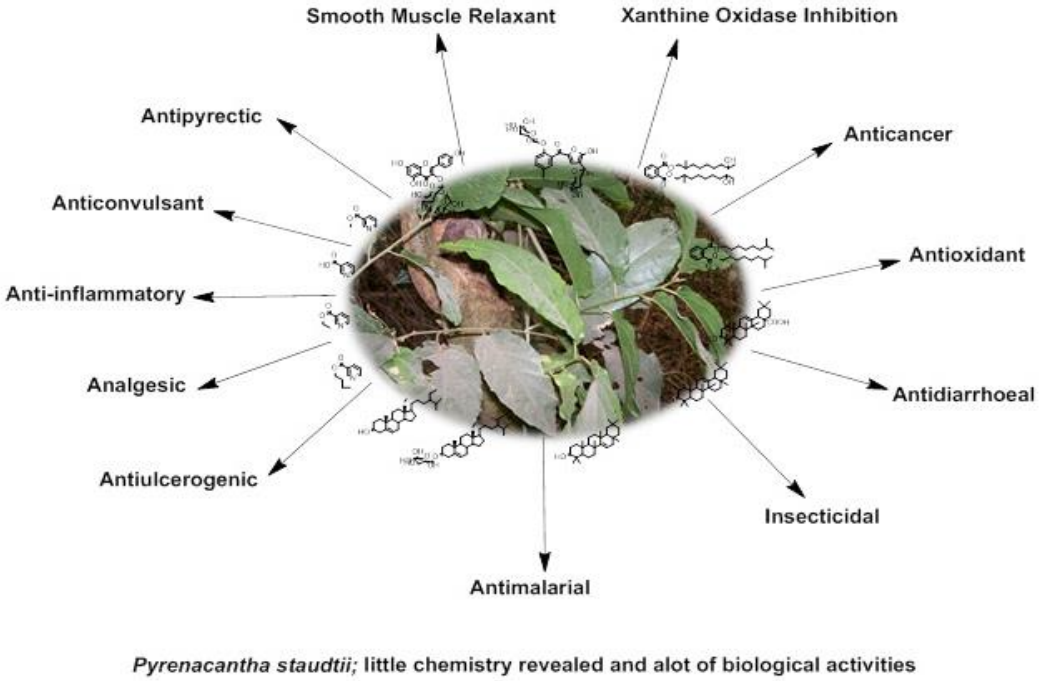

Figure 1: A schematic of *P. staudtii*, some isolated compounds and biological activities.

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 [20].

Antiulcerogenic activities

The antiulcer activities of the aqueous leaves extract of *P. staudtii* against ulcers induced by different experimental models: aspirin, indomethacin, serotonin, reserpine [4], 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 extracts exhibited 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 [5, 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 attributable 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 [4]. Whereas cimetidine did not prevent reserpine induced ulcers, instead it potentiates the ulcerogenic effect of reserpine, the ulcer index increasing from 0.90 ± 0.29 to1.38 ± 0.45. Akubue *et al*. [6] reported the significant ($P < 0.05$) reduction of incidence of histamine-induced gastric ulcers in rats as described by [35] following the oral administration of 1 mL of aqueous extract of *P. staudtii*.

Different workers [74-75] reported the gastric mucosa protective activities of different medicinal plants. These studies agree with that of Aguwa and Mittal [5].

Anti-inflammatory, analgesic activity and antipyretic activity

The *in vivo* analgesic effects of the aqueous leaf extract of *P. staudtii* was reported [20]. 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 prostagladins (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 stools excreted by each animal in 6 h, as well as the total weight of the diarrheic feces in that period of 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 [40].

In the castor oil-induced 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) exhibited 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 at $P < 0.001$.

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 farthest distance of the total length of small 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 ($P < 0.05$–0.01) 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 antimotility 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 antimotility 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 [41] which stimulates the production of several mediator substances that include prostaglandins, nitric oxide, and platelet activating factor, cAMP and tachykinins [42] and by the liberation of prostaglandins by colonic cells [43]. Several mechanisms may be attributed to the antidiarrheal activity of *P. staudtii* extract since it caused inhibition of diarrhea in all the experimental models.

As earlier stated, it could be said that this effect may be due significantly to the inhibition of various mediator substances: prostaglandin, 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 [5, 15] the effect of which is thought to be due to inhibition of calcium mobilization. Reynolds *et al.* [44] proposed that calcium antagonism is responsible in part for the antidiarrheal effect of loperamide and other compounds with calcium-antagonist properties like verapamil and diltiazem which exhibit spasmolytic 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 phytocontistuents acting as calcium antagonist on one hand and by mediation of antimitolity effects by blockade of muscarinic cholinoceptors and/or histaminic receptors on mammalian intestine. Therefore, PS may be exhibiting its antimotility activity by the same pathway as atropine a muscarinic cholinoceptor antagonists which could synergistically potentiate the antidiarrheal effect of *P. staudtii* extract by enhancing the extract's effect on both intestinal secretion and motility.

Anticancer and Cytotoxicity activity

In order to established the ethnomedicinal claims for the use of *P. Staudtii* extract in the treatment of various ailment including cancer Engel *et al*.[16, 17] 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, MTT viability assay, annexin V/propidium iodide (PI) staining, TUNEL method and the determination of apoptotic and adhesion relevant proteins [17]. 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 cells. The rate of apoptosis however was measured by the determination of the sub G1-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 anticancer agent causes biphasic effects on hormone-dependent cancer cells [46] .Genistein at 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.* [47], reported antitumor activities of genistein a soy isoflavone that acts by inhibiting various kinases responsible for the induction of most cancers. Genistein is reported to inhibit protein tyrosine kinase (PTK), matrix metalloprotein (MMP9) and also down-regulate the expression of about 11 genes example VEGF. Through this inhibition and down-regulation of these kinase and matrix-matrix cell adhesion proteins, genistein is able to arrest cell growth, invasion, angiogenesis and cell proliferation at the G2/M. This mechanism is in line with that reported by [16-17] for *P. staudtii*. The plant extract exerts it influence on apoptosis. This was determined by using Alexa Fluor488 Annexin V/PI staining. The treatment with 10 µg/mL *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 rounding of the cells an early stage of apoptosis. β-integrins are mediators of cell-cell and extracellular matrix adhesions which promotes metastasis. This detachment from the extracellular matrix or loss of contact with neighboring cells is a clear indication of induction of an apoptotic process that is termed anoikis [48]. Anoikis 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 the 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 domain 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]. *P. staudtii* has an IC_{50} value of 37.36 µg/mL by these colorimetric methods 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

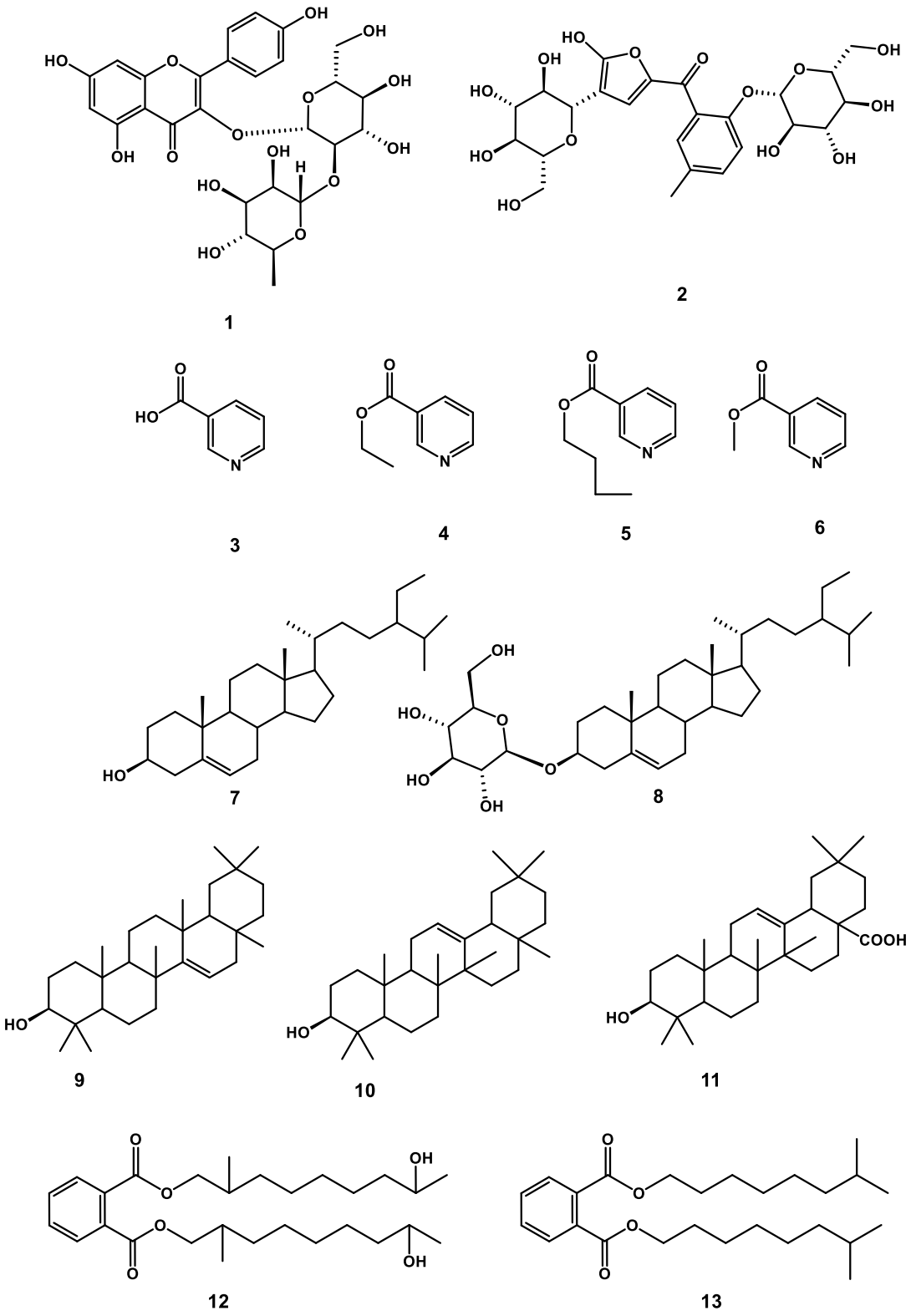

Figure 1: Structures of compounds 1-13 isolated from *P. staudtii*

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. While the extracts produced varying degrees of inhibition of growth in the emerging radicle of the seed of *Sorghum bicolor*. Shweta *et al*., [50] isolated camptothecine a known anticancer drug from the fruits of *Pyrenacantha volubilis* (Icacinaceae) a specie of the genus Pyrenacantha and showed its anticancer activities. They also reported the effectiveness of the seed extracts of this specie against breast cancer, ovarian, colon and carcinoma cell lines (with IC_{50} 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 [16, 17], that members of the family Icacinaceae possess anticancer activities.

Smooth muscle relaxant activity

The smooth relaxant activity of 3-carbomethoxypyridine isolated from *P staudtii* [15] and its derivatives 3-carboethoxypyridine and 3-carbobutoxypyridine were investigated against oxytocin induced and 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-carbomethoxypyridine 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-carmethoxypyridine with subsequent reduction in spontaneous uterine contractions, which was restored after 4 minutes. On the other hand, 3-carboethoxypyridine 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-carboethoxypyridine, spontaneous uterine contractions were restored 1 h after washing. This could be an advantage in threatened abortion, since this compound (3-carboethoxypyridine) abolishes oxytocin induced uterine smooth muscle contractions and spontaneous contractile activity. The inhibitory effect of 3-Carbobutoxypyridine 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-Carbobutoxypyridine unlike the other two agents completely inhibit uterine contractions without any spontaneous activity even at higher doses of oxytocin [14]. The increment in the alkyl 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-carbobutoxypyridine [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 IC_{50} values determined by EZ-FIT windows-based software shows that the two new compounds (kaempherol 3-*O*-*β*-rhamnopyranosyl (1→6)- *β*-D-glucopyranoside and 4-*β*-glucopyranosyl-(2-furyl)-5-methy-l,2-glucopyranoside phenylmethanone) have 23 % and 39 % xanthine oxidase inhibitory effects compared to Allopurinol (IC_{50}, 10.23 ± 2.67). However, the known compound 3-pyridinecarboxylic acid also exhibited some xanthine oxidase inhibitory effect at 68 % (IC_{50} 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 IC_{50} of samples was determined by using EZ-FIT windows-based software. Oxidative stress 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 [51]. Xanthine oxidoreductase (XOR) is part of a group of enzymes known as the molybdenum iron-sulfur flavin hydroxylases first discovered in milk [52] 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 endothelial dysfunction, 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 berghei* in mice. In the *in vitro* assay, the dichloromethane fraction of *P. staudtii* with $IC_{50} < 1$ µg/mL was the most active followed by the aqueous extract IC_{50} 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 castanaeum* (TC) and *Rhyzopertha dominic* (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 1.0191 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[23]. Permethrin is a pyrethriod 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%), 1,8-cineole (14.7%), phallendrene (12.45 %); hexadeconoic acid (28.32 %) which have been shown by different workers to have significant insecticidal activity against rice weevil *Sitophilus oryzae* and *Rhyzopertha dominica* [57]. Linoleic acid has also been reported to exhibit high toxicity against instar larvae with LC_{50} values ranging from 4.78 - 9.11 g/100 mL [58]. Hexadecanoic acid has been shown to exhibit larvicidal effect against *Plasmodium falciparum* [59]. Essential oils of *P. staudtii* could be a lead for insecticidal agent against most store insects.

Acute and Sub-acute toxicities effects

Anosike *et al*. [60] 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 (LD_{50} > 5 g/kg body weight). In the subacute toxicity studies [13], the aqueous extract of *P. staudtii* did not show any significant elevations in the serum concentrations of all the biochemical parameters measured (creatinine, urea, Glutamate-oxaloacetate transaminase (GOT) and Glutamate-pyruvate transaminase (GPT)). It was observed that blood concentrations of the parameters investigated after 30 days of administration of dried aqueous extract of *P. staudtii* were similar to those of the untreated controls. Creatinine levels 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, CCl_4, 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

Table 1: Percentage constituents of the oils of *Pyrenacantha staudtii* bark, root and leaf

Components	Stem Bark (%)	Root Bark (%)	Leaf (%)
α-Thujene	n.d.	trace	0.5
α-Pinene	2.8	2.5	0.5
Camphene	--	--	0.67
β-Myrecene	--	--	3.26
β-Pinene	n.d	0.3	--
α-Phellandrene	0.4	0.1	12.45
Δ^3-Carene	n.d.	0.3	trace
p-Cymene	2.1	0.7	2.42
Limonene	n.d.	1.0	1.25
Terpene	--	--	3.57
Camphor	--	--	0.23
1,8-Cineole	11.1	4.6	--
Linalool	0.2	0.1	--
Nonanal.	n.d	0.1	--
Terpin-4-ol	0.2	n.d.	--
α- Terpenylacetate	--	--	trace
Naphthalene	n.d.	0.1	--
α-Terpineol	0.5	0.1	trace
Methyl salicylate	n.d	0.1	1.35
Decanal	n.d	0.1	--
Citronellol sp.	--	--	7.00
Theaspirane A	0.2	0.2	--
Theaspirane B	0.2	0.1	--
trans,trans-2,4-Decadienal	n.d.	0.1	--
α-Copaene	0.3	0.1	--
β-Cubebene	0.2	n.d	--
β-Elemene	0.4	0.1	--
Cyperene	0.1	n.d.	--
Tetradecane	n.d.	0.1	--
β-Caryophyllene	2.3	0.2	5.57
trans-α-Ionone	0.8	0.1	--
trans-α-Bergamotene	0.1	n.d.	--
Dihydro-β-Ionone	0.2	n.d.	--
Dihydro-β-Ionol	0.1	n.d.	--
α-Humulene	0.6	0.1	--
Geranyl Acetone	1.9	0.4	--
Alloaromadendrene	0.6	n.d.	--
7-Methoxy-2,2-dimethyl-2*H*-1-benzopyran	0.5	0.2	--
Germacrene-D	0.2	n.d.	--
ar-Curcumene	1.0	0.1	--
β-Ionone	1.1	0.2	--
Valencene	0.3	n.d.	--
2-Tridecanone	0.2	n.d.	--
α-Muurolene	0.3	n.d.	--
Cuparene	0.2	n.d.	--
β-Bisabolene	0.2	n.d.	--
γ-Cadinene	n.d.	0.1	--
δ-Cadinene	0.5	0.1	--
α-Calacorene	0.2	n.d.	--
Elemol	0.2	n.d.	--
trans-Nerolidol	0.5	n.d.	--
Spathulenol	1.4	0.4	--
Caryophyllene oxide	4.0	0.5	5.54
Viridiflorol	0.3	0.1	--
Hexadecane	n.d.	0.1	--
1,10-di-*epi*-Cubenol	1.0	n.d.	--
Dill Apiole	0.4	n.d	--
Caryophylla-4(12),8(13)-diene-5β-ol	0.6	n.d.	--
τ-Cadinol	0.9	0.1	--
β-Eudesmol	0.3	n.d.	--
α-Cadinol	0.8	0.1	--
Cadalene	0.6	0.1	--
Acorenone	0.9	0.1	--
Tetradecanoic acid	0.5	1.2	20.60
6,10,14-Trimethyl-2-pentadecanone	1.6	n.d.	--
Pentadecanoic acid	1.1	4.8	--
Farnesyl Acetone C	4.5	0.8	--
Hexadecanoic Acid	38.7	63.0	28.32

treatment of liver disorders could also cause further damage to the liver [62, 63]. Hepatoprotective effects and activity of ethanol extracts of *P. stuadtii* leaves have been reported by various workers [13, 60]. In one study [60], single dose carbon tetrachloride (CCl_4) 5 mL/kg was injected I.P. to the rats in the test group followed by 750mg/kg and 1500 mg/kg body weight of plant extract 48 h later except the positive control group. All the animals were sacrificed day 5 of the experiment and different biochemical parameters investigated. Results from the study showed that at the concentrations (750 mg/kg and 1500mg/kg body weight) of the extract given, there was a significant dose dependent reduction ($P < 0.05$) in CCl_4 induced elevations in the liver enzymes; alanine aminotransferase (ALT) and Aspartate aminotransferase (AST). Also observed are lower values of total and conjugated bilirubin. There was a reduction in the AST from to 127.25 ± 14.84 in the untreated positive control group to 88.50 ± 8.54 and 86.25 ± 13.32 for extract doses of 750 mg/kg and 1500 mg/kg body weight, respectively. Similarly, levels of ALT were reduced from 42.50 ± 9.71 (untreated group) to 26.00 ± 5.66 and 21.00 ± 3.16 at doses given. It is of note that elevation of liver enzymes and bilirubin (a major breakdown product of hemoglobin) in the bloodstream are diagnostic measure of liver damage as a result of cellular necrosis and cell membrane permeability [64]. Liver damage and obstruction of the bile duct have been reported [64] to be responsible for increased levels of total bilirubin as a results of decreased liver uptake and its conjugation. The study clearly revealed that *Pyrenacantha staudti* extract mitigates increase levels of AST, ALT, total and conjugated bilirubin which suggests the hepatoprotective effects of the extract against CCl_4 induced liver toxicity and could be used in the management of liver damage. The hepatoprotective activity of plants *Gracilaria edulis* [65]; *Laurencia obtusa* and *Caulerpa prolifera* [66] extracts also supports the studies of Anosike *et al*. [60] and Mesia *et al.* [13]. It therefore follows that herbal products with no or less side effects as in the case of *P. staudtii* could be used to manage this condition.

Antioxidant

Reactive oxygen species are implicated in inducing oxidative damage to human body, cardiovascular diseases, cancer, liver cirrhosis arteriosclerosis, gout and inflammations. Antioxidants are the compounds that sequestrate ROS and reduce the risk of these diseases [67]. Several studies have linked plants phytoconstituents (polyphenols, flavonoids, tannins, alkaloids), their antioxidant activities to their protective effects against oxidative stress and hepatic cell damage [68-72]. Most of the biological activities reported in this plant have been linked to the presence of phytochemicals that exhibits antioxidant effects in ameliorating disease conditions in man. This is inferred by Anosike *et al*. [60] and Mesia *et al.* [13].

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, concoctions and pastes) as remedy for diseases affecting man, such as cancer, malarial, inflammations, ulcers, blennorrhea, 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 alkaloids, tannins, triterpenoidal saponins, glycosides, carbohydrates, reducing sugars, flavonoids, resins. Extracts and fractions from different parts of the plant have exhibited numerous pharmacological effects in experimental animals and *in vitro* studies such as anticancer, anticonvulsant, antiulcerogenic, hepatoprotectives, insecticidal, analgesics and tocolytics. Flavonoids, triterpenes, sterols and pyridine like compounds have been isolated and characterized from this plant. These findings seem to validate the use of this plant in folkloric medicine and also form a basis for further and detailed investigations in order to tap the therapeutic potentials of the plant towards drug discovery.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article are original and that any liability for claims relating to the content of this article will be borne by them.

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