British Journal of Pharmaceutical Research
12(2): 1-18, 2016, Article no.BJPR.26689
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Glyphaea brevis (Spreng.) Monach.: A Review of the Ethno-medical, Phytochemical and Pharmacological Investigations

Newman Osafo[1*] and Yaw Duah Boakye[2]

[1]Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[2]Department of Pharmaceutics, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Authors' contributions

This work was carried out in collaboration between both authors. Authors NO and YDB designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author NO managed the literature searches and author YDB proof read the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2016/26689
Editor(s):
(1) Q. Ping Dou, Barbara Ann Karmanos Cancer Institute, Departments of Oncology, Pharmacology and Pathology, School of Medicine, Wayne State University, USA.
Reviewers:
(1) Ali Talha Khalil, Quaid-i-Azam University, Pakistan.
(2) Cimanga Kanyanga Richard, University of Antwerp, Belgium.
(3) S. M. Khasim, Acharya Nagarjuna University, Andhra Pradesh, India.
Complete Peer review History: http://sciencedomain.org/review-history/15040

Review Article

Received 28th April 2016
Accepted 8th June 2016
Published 16th June 2016

ABSTRACT

Ethnopharmacological Relevance: *Glyphaea brevis* (spreng) Monach. belongs to the family Tiliaceae. Traditionally it is used in Africa and South America to treat various disease conditions of man including fevers, gonorrhea, dysentery, stomach troubles, lung troubles, parasitic infections, convulsions and constipation. In recent years, it has come under the lime light of researchers in various parts of the world due to its broad ethno-medicinal uses. The aim of this review is to highlight the folkloric significance, phytochemical composition and reported pharmacological activities of *G. brevis.*

Materials and Methods: Google Scholar, Excerpta Medica database and PubMed, were the electronic databases used to search for and filter published research on *Glyphaea brevis*.

**Corresponding author: E-mail: nosafo.pharm@knust.edu.gh;*

Results: The review captures significant data from published literature on the ethno-botanical uses of *G. brevis* which spans from 1985 to 2014. *G. brevis* crude extracts and phytochemicals showed a wide spectrum of activity including anti-infective, antioxidant, anti-allergic, anti-inflammatory, anticonvulsant, anti-proliferative, hypocholestrolaemic activity, weight control and blood glucose control activity, glycosidase inhibiting activity and hepatoprotective effect. However, *G. brevis* is reported to be toxic in reproductive studies.

Conclusion: On the bases of some of these reported biological activities of *G. brevis*, crude extracts and phytochemicals from the plant will require further studies to ascertain the mechanisms of action, potential product development and possible future clinical trials to serve as alternative therapy.

Keywords: Glyphaea brevis; ethno-medicine; phytochemistry; pharmacology; review.

1. INTRODUCTION

The shrub *Glyphaea brevis* (Spreng) Monachino of the family Tiliaceae was selected for this review. *G. brevis* which is an integral part of folkloric medicine in most parts of Africa and South America is used traditionally to manage various ailments such as fevers, gonorrhea, dysentery, stomach troubles, lung troubles, parasitic infections, convulsions, constipation, insect control, etc [1-3]. In the past few years, there has been tremendous increase in research on *G. brevis* which has led to the identification of its anti-infective [4,5], antioxidant [6], anticonvulsant [7], anti-inflammatory [5,8], antiproliferative [9], hepatoprotective effects [10], etc. and a subsequent discovery of some lead compounds of therapeutic importance. However, to the best of the knowledge of the authors, there is no existing review on the reported biological and pharmacological activities of this plant This has called for the need to compile all available data on *G. brevis*. Hence, this review seeks to highlight the folkloric significance, phytochemical composition, pharmacological and various biological activities of *Glyphaea brevis*. The review might also provide an opening for future studies aimed at isolation, purification and characterization of bioactive compounds responsible for the reported biological activities inherent in this plant.

2. HISTORICAL PERSPECTIVE

Glyphaea brevis has been indexed in a lot of ethno-botanical research articles. It is used in folklore medicine for a wide range of conditions and has been well documented in a number of research articles. Aside its ethnopharmacological uses (Table 1), *G. brevis* is widely employed in a number of Agri-horticulture practices such as ornamental usage. In partially tended Agri-horticulture and cultivated uses, it is used as hedge and markers as well as fodder. The wood of *G. brevis* is used as building material and the fiber from the stem employed in farming, forestry, hunting and fishing. The twig is chewed as teeth cleanser and the leaf employed in religious and superstitious ceremonies [1,11].

Table 1. Ethnopharmacological uses of various parts of *Glyphaea brevis*

Plant part	Ethnopharmacological uses	References
Leaf, flower	Food	[1,3]
Leaf	Analgesic, vermifuge, diuretic, abortifacient, ecbolic, hepatitis, dyspepsia, ulcer, gonorrhea	[1,2]
Leaf, bark	Pulmonary troubles	[3,7]
Twig	Stomach troubles	[1]
Root	Antiemetic, veneral diseases, antidote (stings, bites, etc), insecticide, arachnicides, aphrodisiac, appetizer, laxative, sleeping sickness, chest pains	[1,2]
Fruit-pulp	Paralysis, epilepsy, convulsion, spasm	[1,3]
Leaf, root, flower, fruit	Cutaneous, subcutaneous parasitic infections, genital stimulant/depressant	[1]
Root, fruit	Diarrhea, dysentery	[1]
Leaf, twig	Febrifuge, dropsy, swellings, oedema, gout medicine	[1]

3. TAXONOMY AND LOCAL NAMES

Classification
Kingdom: Plantae
Phylum : Magnoliophyta
Class : Magnoliopsida
Order : Malvales
Family : Malvaceae
Genus : *Glyphaea*
Species: *Glyphaea brevis*

Fig. 1. The flower and leaves of *Glyphaea brevis* (Image adapted from © Jan De Laet, plantsystematics.org)

It is known in vernacular across cultures worldwide some of which have been noted in this review (Table 2).

Table 2. Vernacular names of *Glyphaea brevis*

S. no	People	Vernacular name	References
1	Igbo	Aloanyasi	[12]
2	Yoruba	Atori	[12]
3	Hausa	Dorina	[12]
4	Ashanti	Foto	[13]
5	Fula-Tukulor	Kɛki	[14]
6	Non	Bola pane	[14]
7	Serer	Mbarambaram	[14]
8	Wolof	Keng	[14]
9	Gola	Niki-guii	[14]
10	Kissi	Lamdẽŋ	[14]
11	Kono	Gbondo-kpokwe Domba-bɔnduɛ Kɔikɛ	[14]
12	Limba	Bulontokawari	[14]

4. ECOLOGY AND BIOGEOGRAPHY

Glyphaea brevis is a spreading shrub, climber or small tree up to 8 m high. It is very common in undergrowth of closed forest, secondary jungle and on river-banks, lowlands to sub-mountain and wide spread in tropical Africa [11]. It is widely distributed in Africa and South America. It is considered a vegetable in some cultures [15].

5. PHARMACOGNOSTIC DATA

Glyphaea brevis is a tall, often straggling; branchlets sparsely stellate-puberulous, later glabrous shrub. Leaves ovate, obovate, obovate-lanceolate or oblong to elliptic, 5–25 cm long, 1.5–14 cm wide, long-acuminate at the apex, the acumen up to 3 cm long, rounded to subcordate at the base, subentire or doubly-toothed, thin, nearly glabrous or sparsely puberulous on the venation above, and pressed puberulous beneath, often with 2 sorts of stellate hairs; lateral nerves in 5–7 pairs with the 2 basal reaching over half way up the blade or beyond; stipules lanceolate, approximately 2 mm long, very deciduous; petiole 1.5–3.5 cm long. Peduncles, 0.5–2 cm long; pedicels, 1–3.3 cm long (Fig. 1). Flowers up to 4.5 cm in diameter; sepals green, yellow inside, oblong, 1.5–2 cm long, 0.3–0.5 cm wide, tomentellous outside; petals golden or lemon-yellow, oblanceolate or narrowly oblong, 1–2 cm long, 4–4.2 mm wide; stamens yellow; style 6 mm long; stigma green (Fig. 1). Fruit brown, spindle-shaped, 3.5–7.6 cm long, 1.2–1.6 cm wide, ridged and beaked. Seeds irregularly ellipsoid, 4 mm long, 3 mm wide, wrinkled when dry [16].

6. CHEMICAL COMPOSITION

Research into identification and characterization of biologically active compounds from higher plants with ethnopharmacological benefits in the treatment of various disease conditions is always being carried out. As such, host of phytochemical investigations by different teams have been done on *G. brevis*. Phytochemical analyses of the aqueous and ethanol extracts of the stem bark revealed the presence of alkaloids, flavonoids, anthraquinones, saponins, glycosides, steroids, phlobatanins and carbohydrates [17]. Ngumah et al. [4] also showed that the ethanol extract of the leaf contained saponins and glycosides.

Chemical investigation of the leaf extract of *G. brevis* lead to the identification of steroidal compounds, triterpenes, and polyols by Mbosso et al. [18].

7. ANALYTICAL TECHNIQUES

There is an ongoing search into finding bioactive compounds from medicinal plants and *G. brevis*

has not been an exception considering its vast application in a number of afflictions of man as well as scientific credence to back some of its uses.

HPLC analytical method was developed and validated to obtain easily performable method for standardizing *G. brevis* extract. The analytical HPLC used was equipped with UV/vis detector set at 280 nm, a dC18 (5 µm) Atlantis 250 x 2 mm column at 25℃, and a binary gradient composed of solvent A (HCO_2H/H_2O, 2/98, v/v) and solvent B ($CH_3CN/HCO_2H/H_2O$ 80/2/18, v/v/v). The column flow rate was set at 0.25 ml min^{-1}.

Upon analyzing the n-butanol extract of *G. brevis* by HPLC at 280 nm, a spectrum was obtained (Fig. 2) and a UV/vis spectra of each HPLC peak were then establish [9]. The peak observed in the studied sample at a retention time of 7.7 min at 280 nm shows two maxima (258 and 293 nm) which are characteristic of protocatechuic acid (Fig. 3). The max absorption of the UV/vis spectra of the compound corresponding to the HPLC peak at RT = 7.7 min matched correctly with the maxima absorption found in the UV/vis spectra of the protocatechuic acid standard solution (Fig. 4). The peak observed in the study sample is therefore protocatechuic acid with high probability [9].

From the cyclohexane, acetoether and methanol extracts of *G. brevis*, Mbosso et al. [18] employed chromatographic and spectroscopic techniques to identify and confirm the presence of eleven compounds. A mixture of *n*-alkanes (**1**) as well as a mixture of linear fatty acid esters of aliphatic primary alcohols (**2**) were identified via nuclear magnetic resonance (NMR)-spectroscopy and gas chromatography (GC). NMR spectroscopy was also used to establish a mixture of stigmasterol (**3a**) and *β*-sitosterol (**3b**); mixture of linear aliphatic primary alcohols (**4**) were identified by NMR spectroscopy and GC); oleanolic acid (**5a**) and echinocystic acid (**5b**) [19], mixture of linear fatty acids (**6**) were identified by NMR spectroscopy and GC. Sitosteryl 3-O-β-D-glucopyranoside (**7**) [20] and *meso*-erythritol (**8**) [21] in these extracts were identified by NMR spectroscopy and X-ray diffraction. A mixture of unsaturated linear fatty acid (**9**), mixture of fatty acid esters of diunsaturated linear 1, 2-diols (**10**) [22,23], and mixture of linear diunsaturated fatty acid ethyl esters (**11**) [22,23] were also identified by NMR spectroscopy and GC.

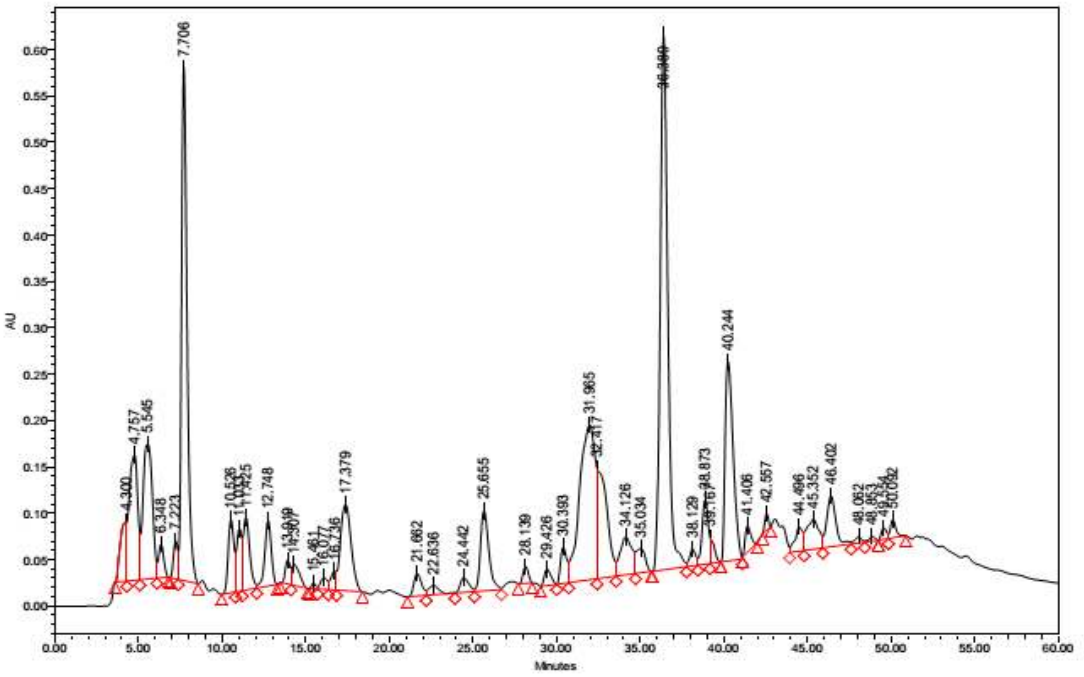


Fig. 2. Chromatogram at 280 nm of n-butanol root extract of *Glyphaea brevis*
(Adapted from Konan et al. [9])

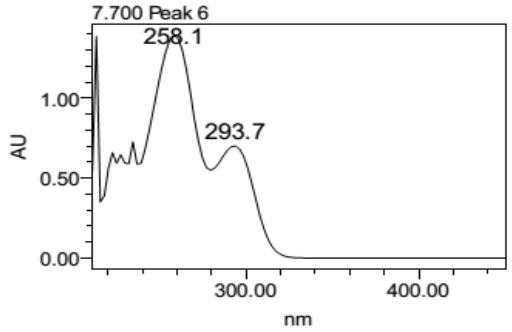


Fig. 3. UV/vis spectrum of the compound at retention time of 7.7 min at 280 nm
(Adapted from Konan et al. [9])

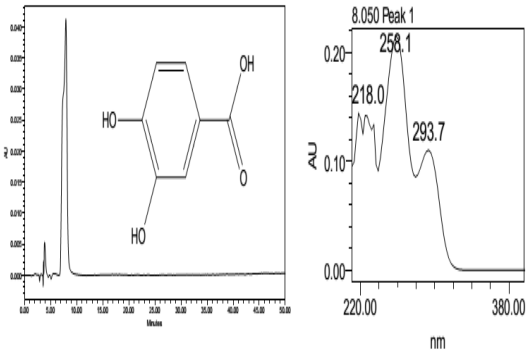


Fig. 4. HPLC and UV/vis spectra of protocatechuic acid (PCA) at 280 nm
(Adapted from Konan et al. [9])

The structures of the above mentioned secondary metabolites were established by spectroscopic analyses, mainly by employing mass spectroscopy and 1D-NMR in conjunction with 2D experimental procedures which included correlation spectroscopy (COSY), heteronuclear multiple quantum (HMQC) and heteronuclear multiple bond correlation (HMBC) spectroscopies and the physical data were then compared with those published. X-ray diffraction or GC were also employed. This study was the first time the linear fatty acid esters of aliphatic primary alcohols (**2**) and *meso*-erythritol (**8**) were being reported [18].

Ekuadzi et al. reported on the presence of epicatechin (**12**) and its dimer procyanidin (**13**) with structures established using spectroscopic procedures [24]. This was the first time these compounds were reported and it confirmed the suspicion of Mbosso et al. of their presence in *G. brevis*.

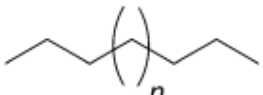
den Hartog et al. reported that erythritol, a polyol, could act as an antioxidant *in vivo* and may also help protect individuals against hyperglycemia-induced vascular damage [25]. Epicatechin and procyanidin showed *in vitro* anti-bacteria and free radicals scavenging activity from further studies conducted by Ekuadzi et al. [24]. They reduced DPPH free radical [24] which is in agreement with earlier studies by other researchers on these compounds showing both their antibacterial [26-30] and antioxidant effects [31,32].

The ten new phenylalkyl-substituted iminosugars, glyphaeaside (**14**, **15**, **18** and **19**) and glyphaeaside (**16**, **17**, **20**, **21** and **22**) (Fig. 5), glyphaeaside C (**23**) and the cinnamic acid derivative glucoside (**24**) were isolated from 80% hydromethanolic extract of *G. brevis* roots (Fig. 6). Their structures were elucidated by 1D and 2D NMR analysis as well as by HR-ESIMS. Compounds **14-23** retain a structure which consisted mainly of iminosugar-like core substituted by a di-, tri- or tetra-hydroxylated nine-carbon chain at the pseudo-anomeric position, substituted by a terminal phenyl group [33].

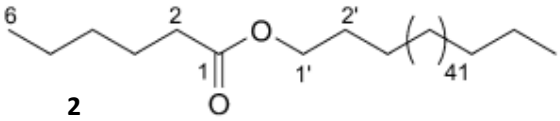
8. PHARMACOLOGICAL ACTIVITY

8.1 Antifungal Activity

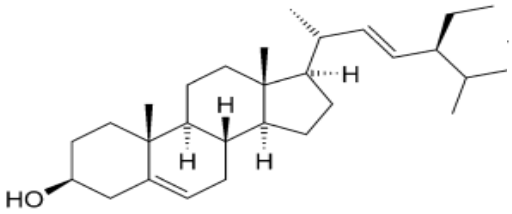
Mbosso et al. reported on the antifungal potential of the hexane extract of the leaves of *G. brevis* from *in vitro* studies conducted [34]. Again, Ngumah et al. investigated the sensitivity of selected fungal pathogens implicated in dry rot of postharvest yam to the leaf extract of *G. brevis* [4]. By employing the cup-plate method, the sensitivity of cold (extracted at room temperature) and hot (extracted at 60℃) ethanol leaf extract of *G. brevis*, against *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium oxalicum* was investigated. The cold ethanol extract showed potency on all test pathogens with a minimum inhibitory concentration (MIC) of 2.24×10^{-3} mg ml^{-1}, 2.24×10^{-3} mg ml^{-1} and 7.08×10^{-3} mg ml^{-1} against *A. niger*, *P. oxalicum* and *F. oxysporum,* respectively. The hot ethanol extract of *G. brevis* yielded an MIC of 4.0×10^{-4} mg ml^{-1}, 3.16×10^{-3} mg ml^{-1} and 1.26×10^{-3} mg ml^{-1}against *A. niger*, *P. oxalicum* and *F. oxysporum,* respectively [4].



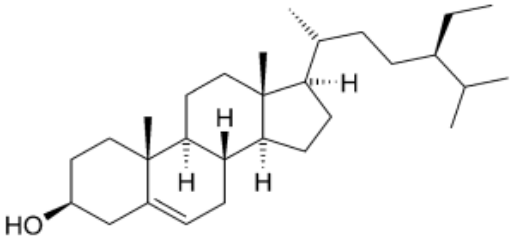
1 (*n* = 14-22)



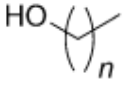
2



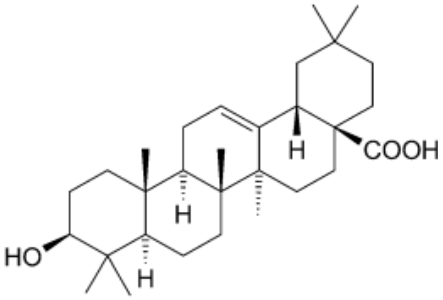
3a



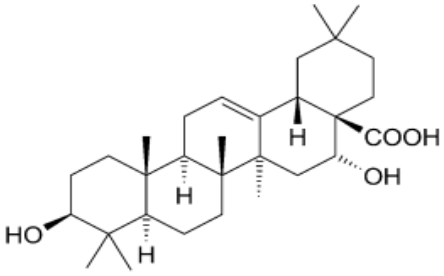
3b



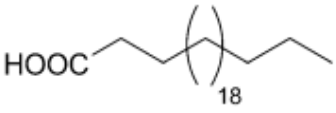
4



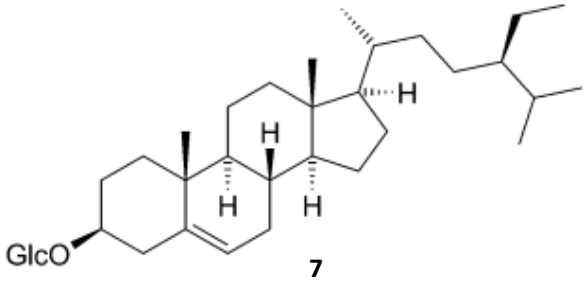
5a



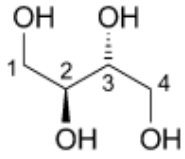
5b



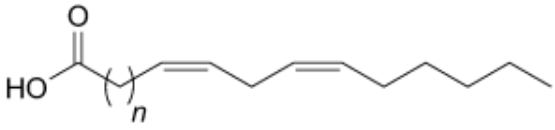
6



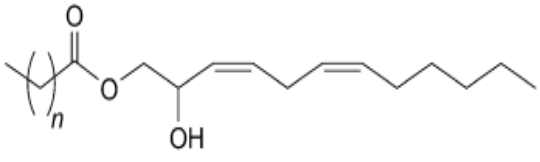
7



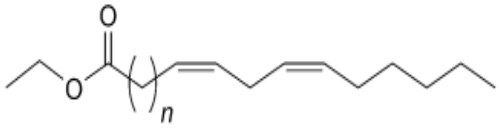
8



9



10



11

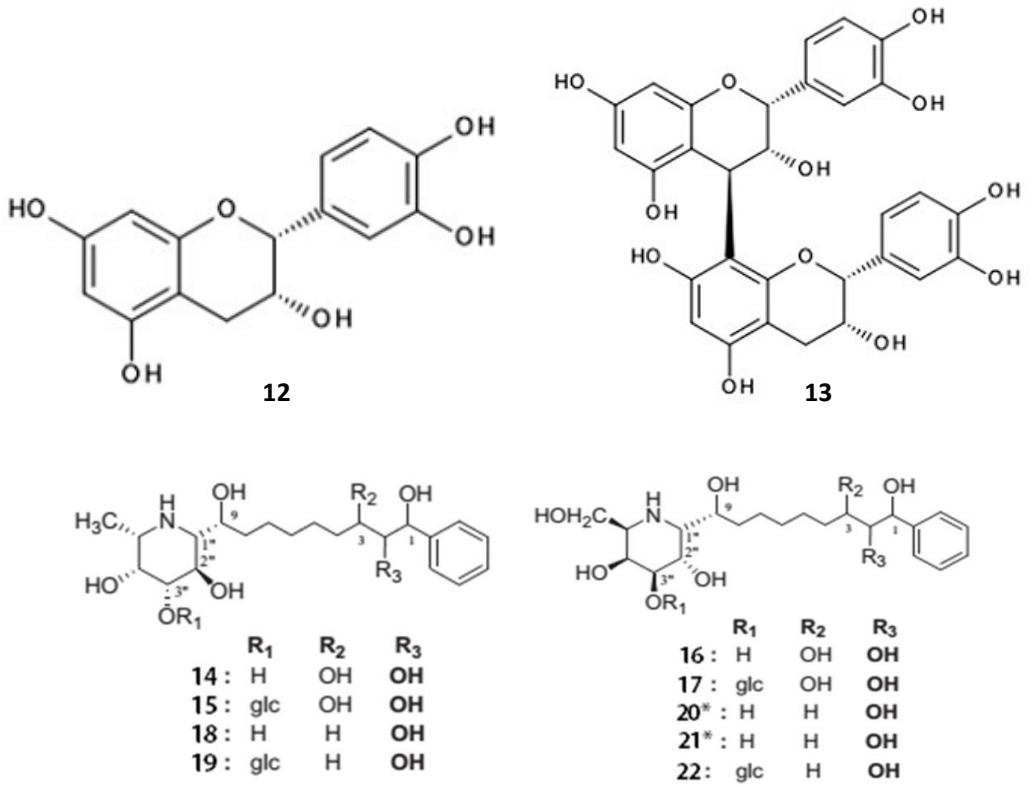


Fig. 5. Structures of secondary metabolites isolated from *Glyphaea brevis*

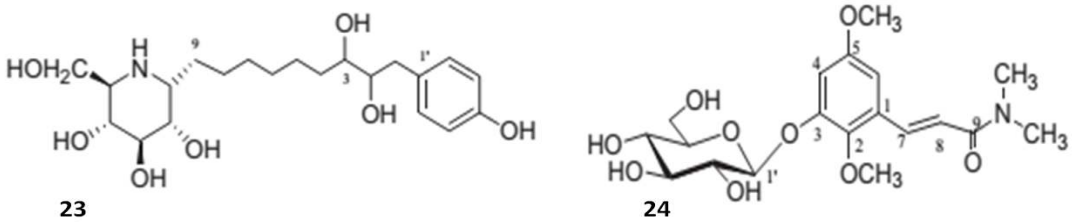


Fig. 6. Structures of alkyl C-iminosugars isolated from the root of *Glyphaea brevis*. *Stereoisomers in C-2 position

8.2 Antioxidant Activity

A couple of studies have assessed the possible utilization of *G. brevis* as a source of phenolic antioxidant. Rapid screening for antioxidant activity of the *G. brevis* extract was done by spotting a methanol solution of the leaf and stem bark extract on silica gel sheet which was developed in chloroform-methanol (9:1 v/v). The developed TLC plate was sprayed with the stable free radical DPPH in methanol. The antioxidant activity of the extract was observed as clear zones against purple background on the developed TLC plates [5].

Using ferric reducing antioxidant power (FRAP), 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS), the antioxidant activity of *G. brevis* was investigated by Dakam et al. [6]. The extraction processes entailed the use of different solvents to extract *G. brevis* to identify the most suitable solvent(s) for maximum yield of phenolics. A two-way extraction protocol that employed the usage of ethanol and/or water was realized to give a high yield of phenolic compounds in the extract. There was a positive correlation between phenolic content of *G. brevis* and observed antioxidant activity measured by the three methods stated [6].

Both the leaf and stem bark extract exhibited antioxidant activities in total phenol content determination, total antioxidant capacity and free radical scavenging activity. The bark extract was higher in phenol contents with very good radical scavenging activity. Both extracts however exhibited similar capacities in the total antioxidant assay [5].

This report on the antioxidant effects of the leaves of *G. brevis* confirms the observations of Dakam et al. in which different assay protocols were employed [6]. However, the total antioxidant capacity, free radical scavenging activity and the total phenol content of the stem bark extract were reported by Dickson et al. [5]. These studies give scientific credence to its folkloric use in the management of oxidative stress and related degenerative diseases.

As reported by Ogbonnia et al. [7] and Dakam et al. [6], the phytochemical screening of the plant revealed the presence of several therapeutically valued constituents including flavonoids and tannins. Therefore, the extract's anti-oxidant activity could be contributed by the presence of flavonoids and tannins which are known to have powerful antioxidant actions [35,36].

8.3 Antibacterial Activity

There was a first report on the possible antibacterial activity of the hexane extract of the leaves of *G. brevis* from an *in vitro* assay [35]. Work done by Oshomoh and Idu established that the aqueous and ethanol extract of the stem bark of *G. brevis* show a significant antimicrobial activity against *Staphylococcus aureus* and *Streptococcus mutans* at concentration of 3.13 mg ml^{-1} [17]. This potent anti-microbial activity of the stem bark makes it suitable as potential agent for dental care and cleansing.

In a separate study conducted by Dickson et al. the leaf extract was realized to show considerable inhibition against some Gram-positive organisms but no inhibition against the Gram-negative organisms tested [5]. The stem bark however showed inhibition against all Gram-positive bacteria tested (i.e. *Bacillus subtilis* NCTC 10073, *Bacillus thurigiensis* ATCC 13838, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212) with the highest inhibition against *B. subtilis and Enterococcus faecalis*. The bark extract also showed activity against the Gram-negatives, *Proteus vulgaris* NCTC 4175 and *Escherichia coli* NCTC 9002. However, *Salmonella typhi* NCTC 6017 and *Pseudomonas aeruginosa* ATCC 27853 were resistant to the bark extract at test concentrations [5].

8.4 Anti-allergic Activity

Anaphylactic response, a severe form of allergic reaction, is reported to be mediated by two pathways: The IgE-dependent pathway with IgE-mast cells as a principal player [37] and the IgG_1 mediated pathway which is chiefly controlled by macrophages and basophils [38]. A drug capable of suppressing the culminating effect of these pathways is said to be a potent anti-allergic agent. Experimentally, compound 48/80 is used as a peptidergic agent to stimulate mast cells and initiate signal transduction pathways involving g proteins [39,40] which results in pro-inflammatory mediators release resulting in systemic allergic response. Studies by Obiri et al. showed that the stem bark extract of *G. brevis* was able to delay the time of mortality (as a results of systemic anaphylactic shock) of mice challenged with compound 48/80 suggestive of *G. brevis*' possible role in stabilizing the lipid bilayer of mast cells hence preventing degranulation [8].

Catalepsy is a condition marked in animals by a fixed posture imposition for a long period of time before regaining of normal posture. It is an extrapyramidal effect of drugs that are able to increase release and activity of histamine in the brain via H_1 receptor [41] or inhibit dopaminergic transmission in the brain via D_2 receptor inhibition [42]. With the identification of histamine containing mast cells in the brain by Schwartz [43] and a reported role of histamine at different stages in catalepsy by Chopra and Dandiya [44], Obiri et al. went further to investigate the possible inhibitory role of *G. brevis* on catalepsy through inhibition of histamine activity by employing the clonidine-induced catalepsy model. Again the role of *G. brevis* in haloperidol-induced catalepsy was investigated to establish the possible role of the extract on dopaminergic transmission leading to catalepsy [8]. They realized from their study that the extract at the prophylactically and therapeutically administered doses of 30, 100 and 300 mg kg^{-1} showed a dose-dependent inhibition of clonidine-induced catalepsy. However, the extract did not inhibit catalepsy induced with haloperidol in both prophylactic and therapeutic protocols suggestive of the fact that *G. brevis* may have no effect on dopaminergic transmission [8].

Considering the extracts inhibitory effect on compound 48/80-induced catalepsy and clonidine-induced catalepsy, it is consistent with earlier findings that extracts with antihistaminic or mast cell stabilizing effect do inhibit clonidine-induced catalepsy [45] as has been reported for *Allium sativum* and *Terminalia belerica*, *Clerodendrum serratum*, *Ficus benghalensis* [46-48], *Xylopia aethiopica* [49] and *Lannea welwitschii* [50].

8.5 Anti-inflammatory Activity

There are a number of studies that have reported the anti-inflammatory activity of *G. brevis* in both acute and chronic inflammatory models. In a study reported by Dickson et al. which reported on the anti-inflammatory action of leaf and stem bark of *G. brevis* in a carrageenan-induced oedema in 7-day old chicks, both extracts significantly reduced the oedema formation at all doses studied [5]. Based on the obtained ED_{50} values for the leaf extract (21.16 mg kg^{-1}) and stem bark extract (21.30 mg kg^{-1}), it was inferred that the extracts have similar potencies though they were found to be four to six times less potent than diclofenac and dexamethasone [5].

To further establish its acute anti-inflammatory potential, Obiri et al. reported on its anti-inflammatory action in mice [8]. In a carrageenan-induced acute inflammatory model in mice, the stem bark extract of *G. brevis* at administered doses of 30, 100 and 300 mg kg^{-1} dose-dependently caused the mean maximal swelling attained during 6 h to be significantly ($p \leq 0.0001$) reduced, respectively, to 30.00±2.84%, 19.56±4.43%, and 19.02±3.36% of the mean inflamed control response of 57.76±2.22%. The total paw swellings induced over the 6 h (measured as the area under the time course curve, AUC) were also dose dependently and significantly ($p \leq 0.0001$) suppressed, respectively, to 67.40±3.55%, 46.89±6.15%, and 35.45±6.70% of the inflamed control response corresponding, respectively, to 32.60±4.33%, 53.11±6.63%, and 64.55±7.15% inhibitions of the mean total oedema response. *G. brevis* administered in the same doses after the induction (curative) of the carrageenan paw oedema significantly ($p \leq 0.0002$) suppressed the mean maximal swelling attained during the 6 h, respectively, to 48.06±1.96%, 43.62±1.10%, and 37.24±2.18% of the mean inflamed control response of 57.76±2.22%. However, the total paw swellings induced over the 6 h were significantly suppressed, respectively, to 87.71±2.23% and 70.55±4.43% of the mean control response at 100 and 300 mg kg^{-1} corresponding, respectively, to 12.29±3.73% and 29.45±5.31% inhibitions of the total oedema response.

It is known that both carrageenan-induced oedema and the primary phase of adjuvant-induced arthritis correspond to those in the early exudative phase of inflammation which is an integral component of the pathology of inflammation [51,52]. Obiri et al. [8] reported on the study on the effect of the stem bark extract of *G. brevis* on rat adjuvant-induced arthritis, a model of chronic inflammation. Adjuvant arthritis was induced in the right hind paw of rats with an intraplantar injection of CFA. Drug effects were evaluated by comparing the maximal and total oedema responses attained during a 28-day period in drug-treated groups with the corresponding values attained in control groups before and after the induction of the oedema on both the ipsilateral (injected) and contralateral (noninjected) limbs.

Daily preventive management over 28 days with *G. brevis* at doses of 30, 100, and 300 mg kg^{-1} produced a significant ($p \leq 0.001$) reduction of the maximal adjuvant-induced swelling 125.08±8.49%, 101.25±7.43%, and 47.75±7.12%, respectively, compared to the mean maximal control swelling of 150.58±9.34%. The total adjuvant-induced response (AUC) over 28 days (polyarthritis) was dose-dependently and significantly ($p \leq 0.0001$) reduced to 76.77±2.93%, 75.05 ± 2.41%, and 42.07 ± 1.92% respectively, of the mean inflamed group response corresponding, respectively, to 23.23±5.02%, 24.95±4.73%, and 57.93±4.50% inhibitions of the total oedema response in the ipsilateral limb. On the acute phase of the arthritic response (day 0–10), the mean maximal oedema response was significantly ($p \leq 0.05$) reduced to 51.80±1.75%, 51.08±1.40%, and 44.87±7.41%, respectively, compared to the mean maximal control response of 66.95±5.42% while the total adjuvant induced response (AUC) over 10 days was significantly ($p \leq 0.002$) reduced to 59.08±1.06%, 59.99±0.97%, and 61.59±5.2% presenting 40.92±7.12%, 40.01±7.11%, and 38.41±8.78% inhibitions of the total oedema response, respectively [8].

On the contralateral (uninjected) hind limb, *G. brevis* stem bark extract administered prophylactically significantly ($p \leq 0.0001$) suppressed the mean maximal oedema swelling in 28 days to 30.76±3.66%, 22.87±4.15%, and

17.12±4.35% relative to the mean maximal control swelling of 92.20±8.95% while the total oedema response was in a dose-dependent manner significantly ($p \leq 0.001$) reduced to 50.14±6.98%, 35.73±2.76%, and 33.62±3.94%, respectively, of the mean control response corresponding to 49.86±8.66%, 64.27±5.83%, and 66.38±6.47% inhibitions of the total oedema response, respectively.

In a separate experiment, *G. brevis* stem bark extract (30, 100 and 300 mg kg-1 daily) was administered beginning from 10 days after the induction of the arthritis (i.e. curative protocol) with daily administration of *G. brevis* stem bark extract at doses of 30, 100, and 300 mg kg^{-1}. On the ipsilateral limb, there was a significant ($p \leq 0.001$) suppression of the mean maximal adjuvant-induced swelling at the end of the 28-day study, respectively, to 109.60±5.03%, 88.41±3.79%, and 67.42±7.91% of the mean maximal control swelling of 150.58±9.34%. The area under the curve (AUC) that represented the total adjuvant-induced swelling during the experimental period was also dose-dependently and significantly ($p \leq 0.05$) suppressed, respectively, to 88.03±2.32%, 78.31±5.09%, and 75.89±1.50% presenting 11.97±4.69%, 21.69±6.52%, and 24.11±4.34% inhibitions of the total oedema response, respectively.

As mentioned earlier, the phytochemical screening of the plant revealed the presence of several therapeutically valued constituents including flavonoids, tannins [6,7] and steroids [18]. Henceforth the extracts anti-inflammatory mechanism may be multifactorial. For example, flavonoids exhibit significant analgesic activity primarily by targeting and inhibiting prostaglandins, PGs [53,54], through inhibition of eicosanoid biosynthesis which are implicated in various immunological responses. Again flavonoids suppress the intracellular Ca^{++} ion elevation and consequently depress the release of pro-inflammatory mediators such as TNFα [55] making flavonoids potent anti-inflammatory agent [56]. Tannins are also documented to be potent inhibitors of cyclooxygenase-1 (COX-1) and with anti-phlogistic activity [57]. Steroids (glucocorticoids) as anti-inflammatory agents act by different mechanisms via the steroid receptor to regulate gene transcription either positively (transactivation) or negatively (transrepression) [58,59]. Again, cross-talk between the steroid and the signaling pathways which are triggered by mast cell activation serves as another mode of their action since some upstream molecules are important in the phosphorylation of transcription factors [60].

8.6 Weight Control and Blood Glucose Control Activity

Obesity is a notable public health concern as it affects a large number of the populace spanning children and adults [61]. This has necessitated the World Health Organisation into classifying obesity as a global epidemic and has emphasized that it is not restricted to industrialised countries [62]. Both environmental and genetic factors have been pinned to the development of obesity. The increase of carbohydrate in Western diets has significant role on the onset of obesity since sugar moieties are substrates for hepatic synthesis of body fat [63,64]. This has made it important to look at inhibition of lipogenesis, through reduction of glucose bioavailability, as a pharmacologic strategy in obesity treatment. Inhibition of the release of glucose from starchy diets through α–amylase inhibition has been a focus of several studies in humans and animals [65,66].

The study by Dakam et al. investigated the possible utilization of *G. brevis* as a novel α-amylase inhibitor [67]. Aqueous and hydroalcohol extracts were prepared from the leaves and their effect on pancreatic α-amylase activity assessed *in vitro* using starch and 4, 6-ethylidene-(G7)-*p*-nitrophenyl-(G1)-α, D- maltoheptaoside (ethylidene-G_7PNP) as substrate. The extracts at doses of 250 and 500 mg kg^{-1} were administered to male albino Wistar rats over a 4-week period and their effects on oral starch tolerance, weight gain and faecal output evaluated. Both extracts exhibited inhibitory effect on α-amylase *in vitro* with the aqueous extract producing 43.09% and 94.59% inhibition against starch and ethylidene-G_7PNP treated groups respectively. The hydroalcohol extract however produced 52.05% and 98.31% inhibition in starch and ethylidene-G_7PNP treated rats respectively. Both extracts again improved oral starch tolerance with a significant ($p < 0.05$) increase in fresh and dry faecal weight coupled with dose-dependent and significantly ($p < 0.001$) reduced weight gain in rats over the period of the study.

These observations suggested that *G. brevis* can constitute a potential source of α-amylase inhibitors which can reduce the dietary starch digestion, glucose bioavailability and henceforth decrease endogenous lipogenesis [67].

8.7 Antimalarial Activity

Malaria is a disease caused by protistan parasite which in severe cases do progress to anaemia, coma or death, especially in children and the elderly [68]. Like all malaria parasites, *Plasmodium berghei* is transmitted by female Anopheles mosquitoes. *P. berghei* has been an important element in the attempt to learn how to manage and eradicate malaria because of the extreme similarity in life cycle and infectious pattern to human infections [69]. The study by Anjuwon et al. investigated the possible *in vivo* antimalarial activity of the methanol extract of *G. brevis* leaves in *P. berghei* infected mice [70].

From their study, they realized that the *n*-butanol, residual aqueous portion and ethylacetate fractions of the methanol extract exhibited suppressive anti-plasmodial activity with 76.64%, 73.25% and 72.99% inhibition respectively [70].

From this study, it can be concluded that *G. brevis* possesses significant anti-plasmodial activity and could offer lead molecules for research and development of new antimalarial therapies.

8.8 Hypocholesterolaemic Activity

Hypercholesterolaemia is a public health concern since it ends up in complications such as hypertension and stroke. The search for innovative, natural and safe treatments to reverse this public health menace henceforth continues unabated. The study conducted by Dakam et al. [71] aimed at evaluating the hypocholesterolaemic activity of *G. brevis* aqueous extract in normal rats as well as streptozotocin-induced diabetic rats.

With daily administration of the aqueous extract of *G. brevis* in both normal and diabetic rats, there was a significant reduction in the levels of fasting blood total cholesterol (normal rats: -38.54%. $p < 0.01$; diabetic rats: -22.08%, $p < 0.01$), LDL-cholesterol (normal rats: -72.85%, $p < 0.01$; diabetic rats: -38.15%, $p < 0.01$). There was however no significant changes in triglycerides. It was realized from this study that there was a significant reduction in the atherogenicity indices total cholesterol/HDL-cholesterol (TC/HDL-c; normal rats: -43.88%, $p < 0.01$; diabetic rats: -32.43, $p < 0.01$) and LDL-cholesterol/HDL-cholesterol (LDL-c/HDL-c; normal rats: -76.14%, $p < 0.01$; diabetic rats: -44.11%, $p < 0.01$) at the end of the study [70].

From this study, there is a scientific evidence of the hypocholesterolaemic effect of *G. brevis*. It can be attributed to the presence of flavonoids that may inhibit the enzyme hydroxymethylglutaryl-CoA (HMGCoA) reductase that are involved in cholesterol biosynthesis [72,73]. This study will therefore provide invaluable data for further research into potent hypocholesterolaemic agent(s) from *G. brevis*.

8.9 Glycosidase Inhibiting Activity

Glycosidases play important role in a number of biological processes, such as digestion, biosynthesis of glycoproteins and lysosomal catabolism of glycol-conjugates. These make glycosidase inhibitors potential agents for the treatment and management of type 2 diabetes, viral infections and cancer [74-76].

The glycosidase inhibitory activity of C-iminosugars isolated from the root extract of *G. brevis* were investigated by Gossan et al. [33] employing *α*-glucosidase (from rice), *β*-glucosidase (from almond), *β*-galactosidase (from *A. oryzae*), *α*-fucosidase (from bovine kidney), *α*-galactosidase (from green coffee beans), *β*-mannosidase (from *H. pomatia*) and *p*-nitrophenyl glycosides in the bioassays. The inhibitory effects of the tested compounds were expressed as the concentration that inhibits 50% of the enzyme activity (IC_{50}). Ten compounds (14–23) were tested at 1 mM against α-glucosidase, β-glucosidase, β-galactosidase, α-galactosidase and β-mannosidase and at 100lM against α-fucosidase.

Compound 23 with a deoxynojirimycin (DNJ) backbone was the most active and showed competitive inhibition with K_i = 31 nM hence the most potent inhibitor of β-glucosidase reported to date [33]. Inhibition of β-mannosidase was observed with compounds 14, 16, 20 and 23 but weak inhibition was realized with the alkyl-C-iminosugars on the other tested glycosidases (*α*-glucosidase, *α*-fucosidase, *α*- and *β*-galactosidase).

From this study, the inhibition potencies of the glyphaeasides isolated from the root extract of *G. brevis* on glycosidase might be modulated by the side-chain which accounts for the binding properties of the glyphaeasides. They do hold potential as therapy for type 2 diabetes mellitus, infections and cancers.

8.10 Anticonvulsant Activity

A study conducted by Ogbonnia et al. [7] aimed at providing scientific data to back the traditional

use of *G. brevis* as an anticonvulsant. This ethnopharmacological activity of the leaves of *G. brevis* was investigated in mice. The strychnine and pentylenetetrazole (PTZ) anticonvulsant evaluations were employed to assess the anti-seizure potential of the extract.

Their study showed that a non-lethal dose of 400 mg kg^{-1} body weight of the extract offered 60% protection to animals against strychnine-induced convulsion. Doses of 400 and 800 mg kg^{-1} of the extract offered the same degree of protection to the animals in the PTZ-induced convulsion. Interestingly, a longer seizure time (8.73±0.8) and shorter seizure time (6.20±1.20) were obtained for 800 mg kg^{-1} and 400 mg kg^{-1} treated rats respectively [7].

From this study, it is evident that *G. brevis* has potential anti-seizure activity which offers scientific credence to its folkloric use. *G. brevis* therefore serves as a possible source of potential anticonvulsant agent.

8.11 Antiproliferative Activity

The *in vitro* antiproliferative activity of *n*-butanol extract of the root of *G. brevis* was investigated by Konan et al. [9] against C6 glioma cells. The cytotoxicity study was performed after 24 and 48 h at a density of 3000 cells per well. After 24 h, the antiproliferative activity was not perceptible; the *n*-butanol extract of the root of *G. brevis* henceforth exhibited low activity against the C6 glioma cells. After 48 h, there was an observable a depletion of the cell line which was gradual and dose-dependent. In their study, the percentage of inhibition of C6 cells did not reach 50% even at *G. brevis* extract concentration of 1 mg ml^{-1}. The n-butanol root extract of *G. brevis* therefore can be said to have a a mild antiproliferative activity. Again, the n-butanol fraction was revealed to be cytotoxic in the C6 glioma cell line which has been reported to be probably due to the presence of protocatechuic acid, a secondary metabolite identified in the n-butanol fraction. Protocatechuic acid has been documented to possess apoptotic potential on human leukaemia cells [77].

8.12 Toxicological Assessment

8.12.1 Hepatoprotective activity

Hepatotoxic effect of cadmium are marked by elevated plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (AP) [78]. Histopathological analysis of liver tissue post cadmium exposure shows that the acute toxicity provokes parenchymal cell necrosis and infiltration of inflammatory cells [79], hepatocellular oedema, sinusoidal congestion, pyknosis and karyrrhexis [78]. These hepatocellular changes do culminate in apoptosis and necrosis as a result of calcium-induced alteration of mitochondrial homeostasis [80]. The study by Ojelabi et al. [10] aimed at investigating the hepatoprotective potential or otherwise, of the ethanol leaf extract of *G. brevis* post cadmium exposure in rabbits.

From their study, they realized that *G. brevis* ethanol leaf extract reduced the toxic effects of cadmium on the liver of rabbits. There was significant ($p < 0.05$) reduction in hepatic marker enzymes with *G. brevis* when compared with the cadmium control rabbits. There was also a significant ($p < 0.05$) increase in plasma albumin, protein and uric acid concentrations [10].

Administration of *G. brevis* clearly showed hepatoprotective activity and it could be attributed to the presence of antioxidant metabolites in the leaf extract [10].

8.12.2 Reproductive toxicity

With male infertility on the rise and various factors are being implicated in these happenings, including exogenous factors. Currently the use of product of natural origin in managing infertility is on the rise. The study by Eweoya et al. [81] aimed at investigating the possible effect of the aqueous and alcohol leaf extract of *G. brevis* on male fertility in adult male Sprague Dawley rats.

The study assessed effect of the extract on testicular function in adult male rats. Sperm count and motility as well as histological parameters were assessed. The alcohol extract at doses of 200 and 400 mg kg^{-1} showed a significant ($p < 0.05$) reduction in the sperm count and motility. Similar observation was realized with 400 mg kg^{-1} of the aqueous extract administered. Both the aqueous and alcohol extract caused a significant ($p < 0.05$) decrease in levels of testosterone but had no significant effect on testicular weight. Histologically, the *G. brevis* extract caused degeneration of seminiferous epithelium and obliteration of interstitial spaces [81].

Though the testicular toxicity cannot be wholly attributed to the *G. brevis* since alcohol is

documented to have toxic effect on Leydig cells and seminiferous epithelium [82], it can be realized from this study that the leaf extract of *G. brevis* has toxic effect on the testis. *G. brevis* henceforth will not be ideal for the management of male infertility.

9. POSSIBLE RESEARCH AREAS ON *Glyphaea brevis*

With a lot yet to be explored on the pharmacological and therapeutic benefits or otherwise of *G. brevis*, it will be prudent that future research considers a number of studies. Some of these are outlined below.

On the antimicrobial potential of *G. brevis*, future research can evaluate the time-kill kinetics as well as establish the mean bactericidal concentration (MBC) of the extract. Also, *in vivo* and *in vitro* antimalarial activity of *G. brevis* should be evaluated using *plasmodium* species that do affect man. More fungal species could also be considered to obtain a broader spectra of its antifungal activity. These could help identify possible compounds that would help augment existing therapy or provide alternative therapies in the management of these infectious conditions.

In vivo antioxidant studies could be conducted on the *G. brevis* extracts since that offers much more important data on its potential antioxidant activity as compared with *in vitro* assays. The anti-allergic activity of *G. brevis* can be affirmed by investigating its mast cell stabilizing activity and possible direct histaminic receptor blockade potential. Exact mechanism of its anti-inflammatory action needs to be elucidated by employing pathway-specific *in vitro* and *in vivo* protocols.

With established potential of *G. brevis* to decrease weight and lower blood glucose, the specific biomolecules involved in its weight lowering potential should be identified. Glyphaesides which have been characterized as glycosidase inhibitor could be further studied as a potential alternative agent in type 2 diabetes mellitus management.

Very specific seizure models should be employed in further studies on *G. brevis*. This will help establish the type(s) of seizures it may be therapeutically beneficial. These would serve as bases for developing or obtaining suitable anti-seizure agents. Also, identifying the specific flavonoid(s) responsible for the hypocholesterolaemic activity of *G. brevis* is relevant. This could serve as a possible alternative HMG-CoA reductase inhibitor for hypercholesterolaemia management.

With the identification of suitable antiproliferative compounds from *G. brevis*, future studies could employ specific cell lines for common cancers of man. The specific mechanisms of anti-proliferative activities of these biomolecules could be established with the aim of obtaining newer and efficacious anti-cancer agents with more tolerable side effect profile.

10. CONCLUSION

From the available data, it is known that *G. brevis* contains alkaloids, flavonoids, anthraquinones, saponins, glycosides, steroids, phlobatanins and carbohydrates. Some of these do account for the observed pharmacologic effect in the conducted *in vitro* and *in vivo* studies on the various fractions and extracts from the different parts of the *G. brevis* plant. Again, a number of studies identified the presence of pharmacologically active secondary metabolites from *G. brevis* by employing spectroscopic techniques. Some of these secondary metabolites had been established to account for the observed responses such as antiproliferative activity, antioxidant and anti-inflammatory activity in *in vivo* and *in vitro* assay procedures.

On the bases of some of these reported biological activities of *G. brevis*, crude extracts and secondary metabolites from the plant will require more clinical trials and product development to be integrated into some of the current treatment protocols as alternative therapy. Since most of the available data limit pharmacological response to the presence of phytochemicals in the plant, it leaves room for further research into mode of action of the extracts and also isolation and identification of lead compounds for research into new drug agents. This will inadvertently lead to discovery of new agents which could possibly have improved pharmacological properties. We can confidently say that *G. brevis* hold a potential of being a source of useful phytochemicals and lead molecules for the drug industry.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Abbiw D. Useful plants of Ghana. Intermediate Technical Publications and Royal Botanic Gardens, Kew, UK; 1990.
2. Mshana NR, Abbiw DK, Addae-Mensah I, Adjanouhoun E, Ahyi MR, Ekpere JA, et al. Traditional medicine and pharmacopoiea. Contribution to the Revision of Ethnobotanical and Floristic Studies in Ghana, OAU/STRC Technical Report. 2000;579.
3. Noumi E, Yomi A. Medicinal plants used for intestinal diseases in Mbalmayo Region, Central Province, Cameroon. Fitoterapia. 2001;72(3):246–254.
4. Ngumah C, Ogbulie J, Orji J. Antifungal potencies of leaf extracts of *Glyphaea brevis* and *Spondias mombin* on fungi implicated in dry rot of postharvest yam. Annals of Food Science and Technology. 2013;14(2):330-335.
5. Dickson R, Ekuadzi E, Annan K, Komlaga G. Antibacterial, anti-inflammatory, and antioxidant effects of the leaves and stem bark of *Glyphaea brevis* (Spreng) Monachino (Tiliaceae): A comparative study. Pharmacognosy research. 2011;3: 166–172.
6. Dakam W, Oben JE, Ngogang JY. Antioxidant activity and extractability of phenolic compounds from medicinal plants: A study of *Glyphaea brevis* Spreng. (Monach.). Pharmacologyonline. 2008;3: 708-718.
7. Ogbonnia S, van Staden J, Jager AK, Coker HA. Anticonvulsant effect of *Glyphaea brevis* (Speng) Moraches leaf extracts in mice and preliminary phytochemical tests. Nigerian Quarterly Journal of Hospital Medicine. 2003;13(3-4):62-64.
8. Obiri DD, Osafo N, Abotsi RE. Antiallergic and antiarthritic effects of stem bark extract of *Glyphaea brevis* (Spreng) monachino (Tiliaceae) in murine models. ISRN Pharmacology. 2013;13.Article ID 874263.
9. Konan KM, Mamyrbekova-bekro JA, Bakalara N, Virieux D, Pirat J-L, Bekro Y-A. HPLC analysis and cytotoxicity of *n* - butanol extract from *Glyphaea brevis* roots against c6 glioma cells. Scientia Pharmaaceutica. 2014;82:171–176.
10. Ojelabi BA, Areola JO, Babalola OO. Roles of *Glyphaea brevis* (Spreng) extract in cadmium induced hepatocellular damage and oxidative stress in rabbit. Journal of Biotechnology and Pharmacy Research. 2012;3(7):123-128.
11. Burkill HM. The useful plants of West Tropical Africa. White Friars Press Ltd. Great Britain. 1985;960.
12. Adegoke EA, Akisanya A, Naqvi SHZ. Studies of Nigerian medicinal plants. IA preliminary survey of plant alkaloids. Journal of West African Science Association. 1968;13:13-33.
13. Owusu-Sekyere E, Cobbina J, Wakatsuki T. Distribution characteristics of mineral elements in tree species from two contrasting secondary forests in Ghana. West African Journal of Applied Ecology. 2006;10:1.
14. Bouquet A, Debray M. Plantes medicinales de la Cote d'Ivoire. ORSTOM 32, Office de la recherche scientifique et technique outre-mer, Paris; 1974.
15. Okafor JC. Agroforestry development in the Boshi Okwango. Division of the Cross River National Park; 1990.
16. Whitehouse C, Cheek M, Andrews SM, Verdcourt B. Flora of tropical East Africa: Tiliaceae and Muntingiaceae. Rotterdam: AA Balkema 120p.-illus. ISBN 902651817X En Icones, Anatomy and morphology, Keys. 2001;2.
17. Oshomoh EO, Idu M. Phytochemical screening and antimicrobial activity of ethanol and aqueous extracts of stem of *Glyphaea brevis* (Spreng.) Monachino on oral microorganisms. Pharmacognosy Journal. 2012;4(33):23-30.
18. Mbosso EJT, Wintjensa R, Lentac BN, Ngouelad S, Rohmere M, Tsamod E. Chemical Constituents from *Glyphaea brevis* and *Monodora myristica*: Chemotaxonomic significance. Chemistry and Biodiversity. 2013;10:224-232.
19. Mahato SB, Kundu AP. ^{13}C NMR Spectra of pentacyclic triterpenoids–a complication and some salient features. Phytochemistry. 1994;37:1517.
20. Ramaiah PA, Lavie D, Budhirija RD, Sudhir S, Garg KN. Spectroscopic studies

on a withanolide from *Withania coagulans*. Phytochemistry. 1984;23:143.

21. Ceccarelli C, Jeffrey GA, McMullan RK. A neutron-diffraction refinement of the crystal-structure of erythritol at 22.6 K. Acta Crystallography. 1980;36:3079-83.
22. Mossoba MM, McDonald RE, Chen JYT, Armstrong DJ, Page SW. Identification of minor C18 triene and conjugated diene isomers in hydrogenated soybean oil and margarine by GC-MI-FT-IR spectroscopy. Journal of Agriculture Food and Chemistry. 1990;38:86.
23. Vlahov G, Rinaldi G, Del Re P, Giuliani AA. ^{13}C nuclear magnetic resonance spectroscopy for determining the different components of epicuticular waxes of olive fruit (*Olea europaea*) dritta cultivar. Analytica Chimica Acta. 2008;624:184.
24. Ekuadzi E, Dickson RA, Fleischer TC, Amponsah IK, Pistorius D, Oberer L. Chemical constituents from *Gouania longipetala* and *Glyphaea brevis*. Natural Product Research. 2014;28(15):1210-1213.
25. den Hartog GJM, Boots AW, Adam-Perrot A, Brouns F, Verkooijen IWCM, Weseler AR, Haenen GRMM, Bast A. Erythritol is a sweet antioxidant. Nutrition. 2010;26:449.
26. Schlingmann G, West RR, Milne L, Pearce J, Carter GT. Diepoxins, novel fungal metabolites with antibioticactivity. Tetrahedron Letters. 1993;34:7225–7228.
27. Caturla N, Vera-Samper E, Villain J, Mateo CR, Micol V. The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. Free Radical Biology and Medicine. 2003;34: 648– 662.
28. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents. 2005;26:343–356.
29. Yang R-Y, Li C-Y, Lin Y-C, Peng G-T, Shea Z-G, Zhou S-N. Lactones from a brown alga endophytic fungus (no. ZZF36) from the South China Sea and their antimicrobial activities. Bioorganc and Medicinal Chemistry Letters. 2006;16: 4205-4208.
30. Cai X, Shan T, Li P, Huang Y, Xu L, Zhou L, Wang M, Jiang W. Spirobisnaphthalenes from the endophytic fungus Dzf12 of *Dioscorea zingiberensis* and their antimicrobial activities. Natural Product Communications. 2009;4:1469–1472.
31. Terao J, Piskula M, Yao Q. Protective effect of epicatechin, epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Archives of Biochemistry and Biophysics. 1994;308: 278–284.
32. Sano M, Takahashi Y, Yoshino K, Shimoi K, Nakamura Y, Tomita I, Oguni I, Konomoto H. Effect of tea (*Camellia sinensis* L.) on lipid peroxidation in rat liver and kidney: A comparison of green and black tea feeding. Biology and Pharmacy Bulletin. 1995;18:1006–1008.
33. Gossan DPA, Magid AA, Kouassi-Yao PA, Behr J-B, Ahibo AC, Djakouré LA, Harakat D, Voutquenne-Nazabadioko L. Glycosidase inhibitors from the roots of *Glyphaea brevis*. Phytochemistry; 2014. Available:http://dx.doi.org/10.1016/j.phytochem.2014.10.029
34. Vinson JA, Dabbagh YA, Serry MM, Jang J. Plant flavonoids, especially tea flavonols, are powerful antioxidants using an in vitro oxidation model for heart disease. Journal of Agriculture and Food Chemistry. 1995;43(11):2800–2802.
35. Brown JE, Rice-Evans CA. Luteolin-rich artichoke extract protects low density lipoprotein from oxidation *in vitro*. Free Radical Research. 1998;29(3):247–255.
36. Mbosso EJT, Ngouela S, Nguedia JCA, Beng VP, Rohmer M, Tsamo E. *In vitro* antimicrobial activity of extracts and compounds of some selected medicinal plants from Cameroon. Journal of Ethnopharmacology. 2010;128:476.
37. Finkelman FD. Anaphylaxis: lessons from mouse models. Journal of Allergy and Clinical Immunology. 2007;120(3):506–515.
38. Miyajima I, Dombrowicz D, Martin TR, Ravetch JV, Kinet J-P, Galli SJ. Systemic anaphylaxis in the mouse can be mediated largely through IgG1 and FcγRIII: Assessment of the cardiopulmonary changes, mast cell degranulation, and death associated with active or IgE- or IgG1-dependent passive anaphylaxis. Journal of Clinical Investigations. 1997;99(5):901–914.
39. Mousli M, Bronner C, Bockaert J, Rouot B, Landry Y. Interaction of substance P, compound 48/80 and mastoparan with the

α-subunit C-terminus of G protein. Immunology Letters. 1990;25(4):355–358.

40. Chahdi A, Fraundorfer PF, Beaven MA. Compound 48/80 activates mast cell phospholipase D via heterotrimeric GTP-binding proteins. Journal of Pharmacology and Experimental Therapeutics. 2000; 292(1):122–130.
41. Jadhav JH, Balsara JJ, Chandorkar AG. Involvement of histaminergic mechanisms in the cataleptogenic effect of clonidine in mice. Journal of Pharmacy and Pharmacology. 1983;35(10):671–673.
42. Sanberg PR. Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors. Nature. 1980;284(5755):472–473.
43. Schwartz JC. Histaminergic mechanisms in brain. Annual Review of Pharmacology and Toxicology. 1977;17:325–339.
44. Chopra YM, Dandiya PC. The relative role of brain acetylcholine and histamine in perphenazine catatonia and influence of antidepressants and diphenhydramine alone and in combination. Neuropharmacology. 1975;14(8):555–560.
45. Dhanalakshmi S, Khaserao SS, Kasture SB. Effect of ethanolic extract of some anti-asthmatic herbs on clonidine and haloperidol-induced catalepsy in mice. Oriental Pharmacy and Experimental Medicine. 2004;4(2):95–99.
46. Vyas BA, Vyas RB. Effect of ethanolic extracts of *Allium sativum* and *Terminalia belerica* on clonidine-induced mast cell degranulation and clonidine and haloperidol-induced catalepsy in mice. International Journal Pharmacy Research. 2009;1(1):41–44.
47. Bhujbal SS, Kumar D, Deoda RS, Kewatkar SM, Patil MJ. *In vivo* and *in vitro* antiasthmatic studies of *Clerodendrum serratum* linn," Pharmacologyonline. 2009;2:745–752.
48. Taur DJ, Nirmal SA, Patil RY. Effect of various extracts of *Ficus bengalensis* bark on clonidine and haloperidol-induced catalepsy in mice. Pharmacologyonline. 2007;3:470–477.
49. Obiri DD, Osafo N. Aqueous ethanol extract of the fruit of *Xylopia aethiopica* (Annonaceae) exhibits anti-anaphylactic and anti-inflammatory actions in mice. Journal of Ethnopharmacology. 2013; 148(3):940–945.
50. Obiri DD, Osafo N, Dei-Anane S, Adjei RK, Ababio EO. Anti-Allergic, Anti-inflammatory and Analgesic Actions of Stem Bark Extract of *Lannea welwitschii* (Anarcadiaceae) in mice. International Journal of Pharmacy and Pharmaceutical Science. 2013;5(4):217-222.
51. Ozaki Y. Antiinflammatory effect of *Curcuma xanthorrhiza* ROXB. And its active principles. Chemistry and Pharmacy Bulletin. 1990;38(4):1045–1048.
52. Silva GN, Martins FR, Matheus ME, Leitao SG, Fernandes PD. Investigation of anti-inflammatory and antinociceptive activities of *Lantana trifolia*. Journal of Ethnopharmacology. 2005;100(3):254–259.
53. Ramesh M, Rao YN, Rao AV, Prabhakar MC, Rao CS, Muralidhar N, Reddy BM. Antinociceptive and anti-inflammatory activity of a flavonoid isolated from *Caralluma attenuate*. Journal of Ethnopharmacology. 1998;62(1):63–66.
54. Narayana KR, Reddy MS, Chaluvadi MR, Krishna DR. Bioflavonoids classification, pharmacological, biochemical effects and therapeutic potential. Indian Journal of Pharmacology. 2001;33(1):2–16.
55. Kempuraj D, Madhappan B, Christodoulou S, Boucher W, Cao J, Papadopoulou N, Cetrulo CL, Theoharides TC. Flavonols inhibit proinflammatory mediator release, intracellular calcium ion levels and protein kinase C theta phosphorylation in human mast cells. British Journal of Pharmacology. 2005;145(7):934–944.
56. Yerima M, Magaji MG, Yaro AH, Tanko Y, Mohammed MM. Analgesic and antiinflammatory activities of the methanolic leaves extract of Securinega virosa (Euphorbiaceae). Nigerian Journal of Pharmaceutical Sciences. 2009;8(1): 47–53.
57. Wagner H. Search for new plant constituents with potential antiphlogistic and antiallergic activity. Planta Medica. 1989;55(3):235–241.
58. Adcock IM. Glucocorticoid-regulated transcription factors. Pulmonary Pharmacology Therapy. 2001;14(3):211–219.
59. Hayashi R, Wada H, Ito K, Adcock IM. Effects of glucocorticoids on gene transcription. European Journal of Pharmacology. 2004;500(1–3):51–62.

60. Gonzalez MV, Jimenez B, Berciano MT, Gonzalez-Sancho JM, Caelles C, Lafarga M, Munoz A. Glucocorticoids antagonize AP-1 by inhibiting the activation/ phosphorylation of JNK without affecting its subcellular distribution. Journal of Cell Biology. 2000;150(5):1199–1207.

61. Bovet P, Chiolero A, Paccaud F. Epidemiology and prevention of obesity in children and adolescent (in French). Revue Medicale Suisse. 2008;4(148):650-652, 654-656.

62. World Health Organisation. Obesity; preventing and managing the global epidemic; report of a who consultation on obesity. World Health Organisation, Geneva, Switzerland. 1997;16.

63. Hudgins LC, Hellerstein MK, Seidman CE, Neese RA, Tremaroli JD, Hirsch J. Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. Journal of Lipid Research. 2000;41:595.

64. Foufelle F, Ferre P. Nutrition, fat synthesis and obesity. In: Remacle C, Reusens B (Eds) Functional Foods, Ageing and Degenerative Disease. CRC Press Boca Raton, FL. 2004;260-277.

65. Unoren J, Kies C. Commercial soybean starch blocker consumption: Impact on weight gain and on copper, lead and zinc status of rats. Plant Food. Human Nutrition. 1992;42:135-142.

66. Thom E. A randomized, double-blind, placebo-controlled trial of a new weight-reducing agent of natural origin. Journal of International Medicine Research. 2000;28: 229-233.

67. Dakam W, Kuate D, Azantsa B, Oben J. Inhibitory effects of *Glyphaea brevis* Spreng. (Monach.) on Pancreatic *α*-Amylase Activity: Impact on Postprandial Blood Glucose and Weight Control in Rats. International Journal of Biomedicine and Pharmaceutical Science. 2009;3(1):5-10.

68. World Malaria Report summary. World Health organization. Accessed 9th December 2012.

Available:http://www.who.int/malaria/world_malaria_report_2012/malaria2012_summary_keypoints_en.pdf

69. Janse CJ, Ramesar J, Waters AP. High-efficiency transfection and drug selection of genetically transformed blood stages of the rodent malaria parasite *Plasmodium berghei*. Nature Protocol. 2006;1:346-356.

70. Anjuwon TM, Ibrahim S, Nok AJ. Evaluation of *in-vivo* antimalarial activity of methanol leaf extracts of *Glyphaea brevis* in *Plasmodium berghei*-infected mice. Tropical Journal of Pharmacy Research. 2015;14(10):1837-1842.

71. Dakam W, Ntentie FR, Oben J. Hypocholesterolemic Effects of *Glyphaea brevis* (Spreng.) monach. In normal and streptozotocin-induced diabetic rats. Planta Medica. 2011;77-PJ21.

72. Chen TH, Liu JC, Chang JJ, Tsai MF, Hsieh MH, Chan P. The *in vitro* inhibitory effect of flavonoid astilbin on 3-hydroxy-3-methylglutaryl coenzyme A reductase on Vero cells. Zhonghua Yi Xue Za Zhi (Taipei). 2001;64(7):382-387.

73. Min SW, Kim DH. Kakkalide and irisolidone: HMG-CoA reductase inhibitors isolated from the flower of *Pueraria thunbergiana*. Biology and Pharmacy Bulletin. 2007;30(10):1965-1968.

74. Kato A, Hollinshead J, Yamashita Y, Nakagawa S, Koike Y, Adachi I, Yu C-Y, Fleet GWJ, Nash RJ. Ana-glucoside of 1,4-dideoxy-1,4-imino-D-lyxitol with an eleven carbon side chain. Phytochemistry Letters. 2010;3:230–233.

75. Mehta A, Zitzmann N, Rudd PM, Block TM, Dwek RA. Alpha-glucosidase inhibitors as potential broad based anti-viral agents. The Federation of European Biochemical Societies Letters. 1998;430:17–22.

76. Wrodnigg TM, Steiner AJ, Ueberbacher BJ. Natural and synthetic iminosugars as carbohydrate processing enzyme inhibitors for cancer therapy. Anticancer Agents in Medicine and Chemistry. 2008;8:77–85.

77. Babich H, Sedletcaia A, Kenigsberg B. *In vitro* cytotoxicity of protocatechuic acid to cultured human cells from oral tissue: Involvement in oxidative stress. Pharmacology and Toxicology. 2002;91: 245–53

78. Dudley RE, Svoboda DJ, Klaassen CD. Acute exposure to cadmium causes severe liver injury in rats. Toxicology and Applied Pharmacology. 1982;65:302-313.

79. Tzirogiannins KN, Panoutsopoulos GL, Demonakou MD, Papadimas GK, Kondyli VG, Kourentzi KT, Hereti RI, Myconiatis MG. The hepatoprotective effect of putrescine against cadmium induced acute

liver injury. Archives of Toxicology. 2004;78:321-329.

80. Lemarie A, Lagadic-Gossmann D, Morzadec C, Allain N, Fardel O, Vercet L. Cadmium induces caspase-independent apoptosis in liver HEP3B cells: Role of calcium in signaling oxidative stress-related impairment of mitochondria and relocation of endonuclease G and apoptosis-inducing factor. Free Radical Biology and Medicine. 2004;36:1517-1531.

81. Eweoya EO, Oyewopo AO, Yama OE. Effect of aqueous and alcohol extract of *Glyphaea brevis* (speng) moraches on the reproductive parameters of adult male sprague dawley rats. West African Journal of Assisted Reproduction. 2011;2(1):16-19.

82. McDonald RM, Smith JF, Montgomery GW, Fleming JS, Cox NR. Sperm DNA damage after scrotal insulation in rams. Proceedings of New Zealand Society of Animal Products. 2007;67:192-197.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/15040