Online ISSN : 2249-4618
Print ISSN : 0975-5888
DOI : 10.17406/GJMRA

Global Journal

of Medical Research: B

Pharma, Drug Discovery, Toxicology & Medicine

Highlights

- Anti-Hyperglycemic and in Vivo
- Phytochemical Screening and in Vitro
- Phytochemical and Ethnobotanical
- Effect on Cellular Antioxidant Defense

Discovering Thoughts, Inventing Future

VOLUME 17 ISSUE 1 VERSION 1.0

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine

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Volume 17 Issue 1 (Ver. 1.0)

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Contents of the Issue

Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 17 Issue 1 Version 1.0 Year 2017
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Phytochemical and Ethnobotanical Study about Tamarisk Gallica in a North Africa South-West of Algeria

By M. M. Elamin
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Abstract- This research is interested in phytochemical valorization of the shrub Tamariskgallica. The phytochemical study of this species has shown their richness in saponosides, steroids, terpenes, and unsaturated sterols revealed the strong presence of polyphenols especially in flavonoids and tannins. These last are widely present in the plant kingdom, and their therapeutic activities.

Keywords: tamarisk gallica, polyphenols, phytochemical study, chemical valorization, the aerial part.

GJMR-B Classification: NLMC Code: QV 19

Strictly as per the compliance and regulations of:

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I. Introduction

The use of medicinal plants as a source of remedy to treat themselves or Prevent diseases is originating in the millennia until the recent Chinese civilization, Indian and the Middle East. It is become certainly. The modern pharmaceuticals industry itself is still supports widely on the diversity of plant secondary metabolites to find new molecules to biological properties unpublished. This source seems inexhaustible since only a small part of the 400000 known plant species have been investigated on plans phytochemical and pharmacological, and that each species may contain up to several thousands of different constituents. Medicinal plants are used mainly in two forms: Complex, containing a broad spectrum of constituents (infusion, essential oils and extracts of the dyes). Pure, chemically defined as active principle. The pure compounds are generally used when the active principles of the plants produce a strong and specific activity or have a low therapeutic index. The Algerian flora with its 3000 species belonging to several botanical families Including 15% endemic, remains very little explored on the phytochemical plan as on the pharmacological plan. The valorization of the Medicinal Plants of the national flora will be a great contribution to the pharmaceutical industry of Algeria and will have an economic impact certain [1][2]. For our part, we have chosen to study the Saharan species *Tamarisk gallica* is part of the family Tamaricaceae, account approximately 50 to 60 species of shrubs to flower in the Tamaricaceae family.

Fig. 1: Tamarisk gallica flowers (side of the Valley of Bechar (south west of Algeria), which located near the district of Djenain Difallah).

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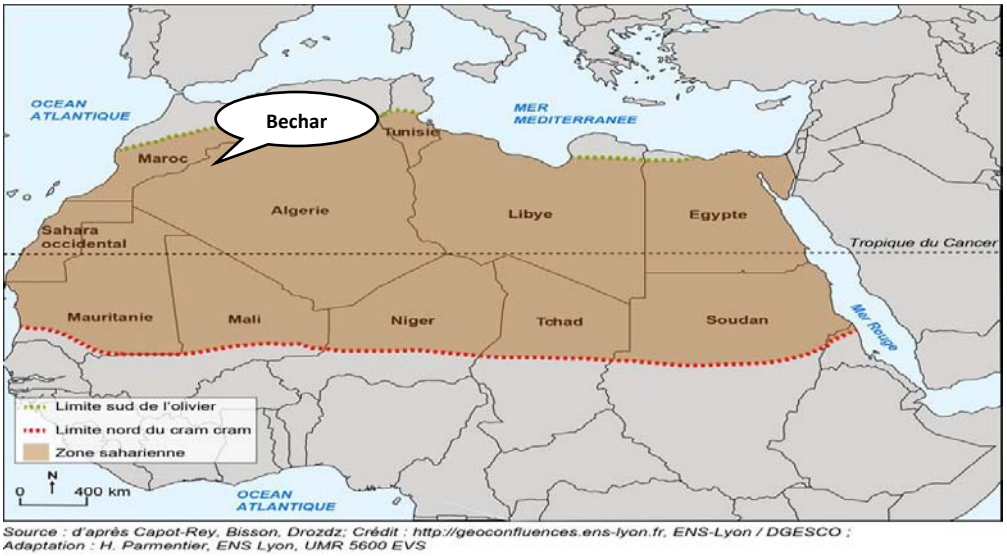
The generic name of origin in Latin is supposed to refer to the river Tamarisk in Spain [3][4]. The Tamarisk are trees or shrubs, frequent in salted land, characterized by small leaves scaly, often nested, and giving the twigs the appearance of those of some junipers.

Fig. 2: Salted land where *Tamarisk gallica* are existing in the west south of Algeria.

The leaves are often punctuated by tiny holes corresponding to funnels at the bottom of which are placed stomata and by where exudes a mucus containing salt and limestone. The roots are in general very developed; their wood contains vessels to large gauge. The flowers are grouped in cylindrical kittens that among some species of genus Tamarisk [5].

Fig. 3: The morphology of leaves and roots of *Tamarisk gallica* shrub of the west south of Algeria.

We know sixty species of Tamarisk capita especially the Mediterranean countries and the South Asia, in dry regions in particular.



Source : d'après Capot-Rey, Bisson, Drozdz; Crédit : http://geoconfluences.ens-lyon.fr, ENS-Lyon / DGESCO ; Adaptation : H. Parmentier, ENS Lyon, UMR 5600 EVS

Fig. 4: The geometrical sites of the existence of *Tamarisk gallica* especially in the north Sahara of Africa.

This kind plays an important role in North Africa and the northern Sahara, where It account about a dozen species of which two are particularly prevalent: T. articulata and T. gallica, designated in Arabic respectively under the names of "Thlaia" (more. "Ethel") and "Fersig" (more "The Aârich") [6].

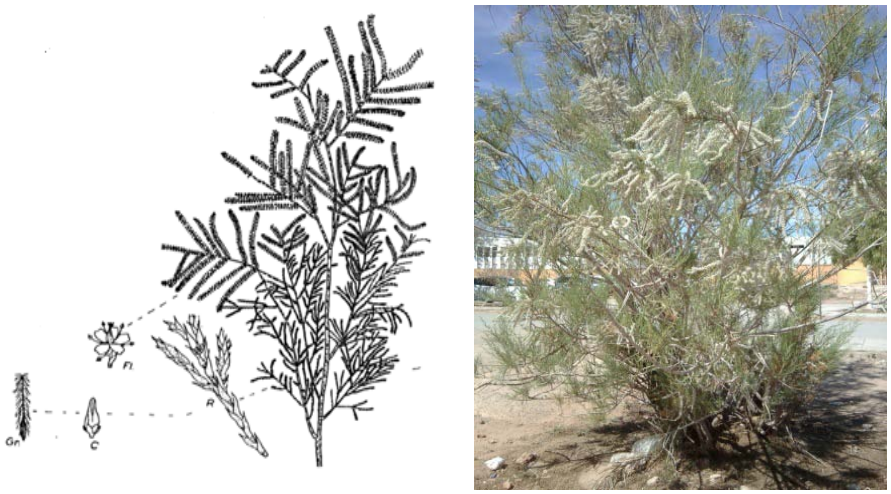


Fig. 5: Morphology of the species *Tamarisk gallica.*

The aim of this work is the identification of the various families of secondary metabolites exist in the aerial part of the species of *Tamarix gallica* following the protocols mentioned below, either by:

- Maceration by using:

1) The diluted hydrochloric acid (5 %) for the identification of flavonoids.
2) Ethanol (70%) for the identification of steroids and sterols unsaturated.
3) Distilled water for the identification of cardenolides.

- Exhaustion by heating by using:

1) Distilled water for the identification of saponosides.
2) Distilled water for the identification of tannins.
3) The chloroform for the identity of the sterols and unsaturated of terpenes.
4) Hydrochloric acid, for the identification of alkaloids.

II. Materials and Method

a) Preparation of plant material

The harvest of the species *Tamarisk gallica* has been carried out the month of February, the 06/02/2015 until 08/02/2015 at the level of the city of Bechar, next to the valley of Bechar, which located near the district of Djenain Difallah. It was the flushing by water ordinal and the drying of the rods with the bark, loads of leaves has been cut away in the dark and dry place for 10-15 days, and thenwe grind them to aid a mill to become in the powder form a little fine, after we retain it in a glass vial well closed.

Fig. 6: The Valley of Djenain Difallah district, Bechar - Algeria (Oued Bechar).

Fig. 7: Leaves and rods grinded of the species *Tamarisk gallica.*

Chemicals: Turnings of magnesium, acetic acid glacial, sulfuric acid, ammonium hydroxide(smoked), chloroform, and ethanol, hydrochloric acid, chloride of iron, methanol, iso-amylic alcohol. Distilled water.

i. *Highlighting of flavonoids (free, glycosides and heterosides)*

The macerate obtained after 48 hours, from a mixture of 10g of plant material and 75 ml HCl (5 %) is filtered.

1.1.1 st Test: detection of flavonoids:

– 10 ml of the filtrate is adjusted to pH (9 to 10) with ammonium hydroxide (smoked). The control of pH has been achieved with the indicator paper pH, which turns dark blue.

⇨ The presence of flavonoids is indicated by the appearance of the color yellow.

Fig. 8: The alkalization of medium with the appearance of the yellow color.

1.2.2nd test: detection of glyosidic flavonoids and heterosidiques:

– The basic solution of the 1st test is brought to the evaporation.

– The residue obtained is dissolved in 3 ml of hydrochloride acid (5 %), and then the mixture (acid extract) is heated slightly.

– After cooling, the acid extract was divided into two fractions.

– Has the 1st fraction, a few seeds of Mg have been added.

⇨ The obtaining of the color grenade attests the presence of flavonoids -glyosidic bonds.

– A quantity of 2.5 ml of alcohol iso-amylic has been added to the 2nd fraction of acid extract.

⇨ The development of the yellow color indicates the presence of flavonoids heterosidiques.

Fig. 9: Formation of the pomegranate color according to the addition of Mg.

1.3.3rd test: detection of free flavonoids:

– Has 10 ml of the filtrate, add 5 ml of alcohol iso-Amylic, the appearance of the color indicates the presence of flavonoids free.

Fig.10: Detection of free flavonoids by the appearance of yellow color.

b) Highlighting of steroids and unsaturated sterols

– The filtrate of the macerate obtained, after 48 h from 3 g of plant material and 20 ml of And ethanol (70 %) is evaporated to sec.

– The residue obtained is dissolved in 15 ml of chloroform.

– The filtrate (the chloroform phase) is subsequently divided into two fractions.

– Has the 1st fraction, it is added up gently on the walls of the tube, an equal volume of sulfuric acid.

⇨ The turning of the color toward the red brick at the bottom of test tube indicates the presence of steroids.

– Has the 2nd fraction of filtrate, was successively added 1 ml of acetic acid and 1 ml of sulfuric acid.

⇨ The persistence (non-disappearance) of the color green indicates the presence of unsaturated sterols.

c) Highlighting of the cardenolides

– A maceration of 24 h, obtained from 3g of plant material and 20 ml of distilled water is filtered. Then 10 ml of filtrate is added to 10 ml of chloroform (liquid-liquid extraction).

– Subsequently, the organic phase is evaporated and the residue obtained is dissolved in 3 ml of acetic acid glacial and then it adds 3 drops of chloride of iron (1 %) and more than 3 ml of sulfuric acid.

⇨ The appearance of the color green-bluish in the acetic phase indicates the presence of cardenolides.

d) Highlighting the saponins

– 3 g of plant material is dissolved in 20 ml of distilled water; the mixture is heated to for 30 minutes at 40°C. After filtration and cooling to ambient temperature, a fraction of the filtrate is introduced in a test tube; this last is agitated manually for 1 minute.

⇨ After 10 seconds of rest, the formation of the foam indicates the presence of the substances saponins. The height of the foam then is measured.

Fig. 11: Formation the foaming layer which indicates the presence of saponosides.

e) Highlighting of tannins

– A mixture of 3 g of plant material with 50 ml of distilled water is heated to 100 °C. The extract is filtered after cooling to ambient temperature.

– The filtrate taken in a test tube adds 3 drops of an aqueous solution of chloride of iron (1 %).

⇨ A cornering of color toward blue-black precipitate indicates the presence of tannins.

Fig. 12: The blue-black precipitate which detects the presence of tannins.

f) Highlighting of the sterols and unsaturated terpenes

– 3g of vegetable matter in the presence of 20 ml of chloroform is heated for 30 min at 40°C. The extract is filtered after cooling to ambient temperature.

– The filtrate taken in a test tube is added slowly to the wall of the tube 1 ml of sulfuric acid.

⇨ The emergence of an intersection of the two phases of green color, which turns into red, reveals the presence of sterols and unsaturated terpenes [7] [8] [9].

g) Highlighting of condensed tannins

Has 5 ml of infused (5g of powder in 100 ml of boiling distilled water); we added 5 ml of concentrated

hydrochloride acid. The assembly has been brought to the boil for 15 minutes and then filter on filter paper of «Watt man ".

⇨ In the presence of tannins catechiques, it will form a red precipitate soluble in alcohol iso-amylic [10].

Fig. 13: The red precipitate which detects the presence of catechic tannins.

h) Highlighting of alkaloids

– A maceration of 2 g of plant material with 20 ml of methanol for 30 minutes.
– The filtrate is evaporated to sec.
– The residue dissolved in 6 ml of chloroform.
– Has the chloroform extract been added a few drops of Reagent of Wagner or Mayer.

⇨ The formation of a turbidity / red precipitate brown indicated the presence of alkaloids [11] [12] [13].

III. Results and Discussion

Table 1: Phytochemical constituents of *Tamarisk gallica*.

Aerial part		
The secondary metabolites	The leaves	The rods
Tannins and condensed tannins	+++	+++
Flavonoids	+++	-
Glycosides flavonoids	++	-
Heterosidiques flavonoids	++	-
Saponins	+	++
Cardinolides	-	-
Sterols and unsatured terpenes	+	+
Steroids and unsatured sterols	-	+
Alkaloids	-	-

'+' Presence, '++' Medium Presence, '+++' Strong Presence, '-'Absent

N.B: the rate of the presence depends on the speed of precipitation

a) Tests of flavonoid (free, heterosidiques and glyosidic)

– The obtaining of a yellow color for the extract of leaves indicated the presence of flavonoids in a part of the plant studied (1st test).
– The obtaining of a yellow color, which thinning passing of sheets, means that there is now of flavonoids heterosidiques (2ndtest).
– Obtaining a color Granada for the extract of sheets indicated the presence of flavonoids -glyosidic (2nd test).
– Obtaining a yellow color of the alcohol phase, for the leaves, which involve the presence of flavonoids free (3rd test).

b) Tests of steroids and unsaturated sterols

– For the leaves, no shift toward red Brown has been found. While the color change yellow to red Brown has been well note for the rods, these variations of color indicates the presence of steroids.
– The disappearance of the dark green color and the appearance of the transparent color, indicate the absence of unsaturated sterols in the leaves. Whereas, the persistence of the green color shows the presence of unsaturated sterols which is significant quantity in the part of the rods.

c) Test of cardenolides

– The obtaining of the red color after the addition of acetic acid glacial to the two parties of the shrub shows the absence of cardenolides.

d) Test of saponins

– In a test tube with a diameter of 1.5 cm, it has measured foam of 2 cm thickness for the part of the rods, by against for the portion of the leaves we noted therefore 1 cm of the foam, which indicates the presence of saponosides.

e) Test of tannins

– A rapid color change toward blue-black follows a precipitate was noted for the two parties' leaves and rods of the shrub this identifies the tannins in all parties. In addition, obtaining a red precipitate in the test of condensed tannins indicates their presence.

f) Test of sterol and unsaturated terpenes

– This test has shown the presence of sterols and unsaturated terpenes. By comparing the two parties. It has been observed, for the leaves the appearance of a layer interphase of color Granada (red-brown) of low thickness. While the rods have shown a strong layer interphase of Grenade color (red-brown).

g) Test of alkaloids

– The test of alkaloids has shown no precipitate for the two parts of the shrub. There are no alkaloids in *Tamarix gallica.*

IV. Conclusion

In view of all these severalresults of phytochemical screening associated with these compounds found in the aerial part of *Tamarix gallica*extract, we recommend further research on this shrub leaves to quantify the concentration of these bioactive compounds per known amount for industrial use. We believe these bioactive compounds in *Tamarix gallica* aerial part shown us could be helpful for pharmaceutical industry and medicinal sciences utilization.

V. Acknowledgments

This research work would not have been possible without the support of many people. The author wishes to express her gratitude to her supervisor, with her assistant who was abundantly helpful and offered invaluable assistance, support and guidance. Special thank also to Faculty of Exact Sciences Laboratory of Tahri Mohammed university bechar, Algeria No. 08000.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 17 Issue 1 Version 1.0 Year 2017
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Relationship between the Wording of the Instructions and the Efficacy of Dapoxetine in the Therapy of Lifelong Premature Ejaculation: A Pilot Study

By Giorgio Cavallini & Carlo Maretti

Abstract- To explore whether medical instructions might improve dapoxetine efficacy for lifelong premature ejaculation (PE).

All PE patients received dapoxetine 60 mg. Sixty patients (Group 1) were given the following instructions “Take one tablet two hours before sexual intercourse; dapoxetine will delay your ejaculation” and 64 patients (Group 2) were given the following instructions “Take one tablet two hours before sexual intercourse; do not try to delay or to hold back ejaculation because dapoxetine is doing it for you. Dapoxetine will delay/improve your ejaculation within 3 months from the first assumption”. Before, and during the course of dapoxetine assumption, the stopwatch-measured intravaginal ejaculatory latency time, the Clinical Global Impression of Change and the side effects were measured in both groups.

Results: The baseline intravaginal ejaculatory latency time and the Clinical Global Impression of Change values did not significantly differ in Groups 1 and 2; however, during the course of dapoxetine administration, they were significantly more improved in Group 2 than in Group 1. The side effects were negligible and did not significantly differ between the groups.

Keywords: premature ejaculation, dapoxetine, medical instructions.

GJMR-B Classification: NLMC Code: QV 752

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Conclusions: Dapoxetine efficacy for the treatment of lifelong PE might be improved by the wording used in the instructions.

Keywords: premature ejaculation, dapoxetine, medical instructions.

I. Introduction

Premature ejaculation (PE) can be a debilitating male sexual impairment. A series of studies has suggested a prevalence of 4–39% (1, 2, 3). This wide range can be partly attributed to variations in the way PE is defined, but may also reflect differences between populations and differences in the degree of annoyance. Furthermore, due to the intimate nature of the problem, PE tends to be under-reported by patients who do not typically seek medical help (1).

Premature ejaculation can be divided into two distinct entities: acquired or lifelong (4). Anxiety and genetic factors are regarded as the most likely causes of lifelong PE (5). We explored the hypothesis that medical instructions (which the Authors consider soothing) might improve the efficacy of a drug.

Dapoxetine is a drug specifically developed for the on-demand treatment of PE. It has been extensively evaluated in five randomized, placebo-controlled phase III clinical trials involving more than 6000 men. There is evidence of its efficacy, its relatively mundane side effect profile and its validity as an on-demand medication. Sixty mg dapoxetine users had their intravaginal ejaculatory latency time (IELT) increased by 3-4 minutes with respect to baseline, and 55-60% were satisfied with its efficacy (6).

This open label, crossover, multicenter, fixed dose, prospective trial explored the hypothesis as to whether medical instructions for the assumption of dapoxetine might improve its efficacy.

II. Materials and Methods

This study was reviewed by the appropriate ethics committee, and all patients gave informed consent prior to their inclusion in the study. This open label, crossover, multicenter, fixed dose, pilot study began on January 2nd 2015 and ended on July 2nd 2016, and was conducted in two centers: Bologna (1st center, Giorgio Cavallini) and Piacenza (2nd center, Carlo Maretti). All patients meeting lifelong PE criteria were considered. Premature ejaculation was defined according to the International Society of Sexual Medicine (7).

1. Ejaculation which always or nearly always occurs prior to or within approximately 1 minute of vaginal penetration;
2. The inability to delay ejaculation during all or nearly all vaginal penetrations;
3. Negative personal consequences, such as distress, annoyance, frustration and/or the avoidance of sexual encounters.

Medical history was collected and a semi-structured interview was carried out; objective examination of the patient involved a general examination as well as a more focused examination of

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the genitalia, namely the scrotal contents and the penis in detail. A digital rectal examination to palpate the prostate gland and a Stamey test were also performed. Endocrine assessment involved thyroid function: total tyroxine (reference range: 60 - 150 nmol/L), free tyroxine (reference range 10 - 25 pmoli/L), total triiodothyronine (reference range 1.1 – 2.6 nmol/L), free triiodothyronine (reference range 3.0 – 8.0 pmol/L) and thyreotropin (reference range 0.15 – 3.5 mU/L) (Wespes et al, 2013).

The exclusion criteria were: congenital penile curvature (3 cases), phymosis (6 cases), short frenum (6 cases), painful prostate at digital rectal examination and/or a positive Stamey test to detect the presence of any level of urinary infection/inflammation (2 cases), thyroid endocrine alterations (no cases), drug and/or alcohol consumption (6 cases) (8), previous or concurrent sexual dysfunction of the patient or of his female partner (0 cases).

The inclusion criteria were: stable heterosexual relationship > 1 year, cohabitation > 1 year and age > 18 years.

The patients were all instructed to take one 60 mg tablet of dapoxetine (Priligy®, Menarini) two hours before sexual intercourse. Two different sets of instructions were given to the patients. The first set of instructions was: "Take one tablet two hours before sexual intercourse; dapoxetine will delay your ejaculation"; the second set of instructions was: "Take one tablet two hours before sexual intercourse; do not try to delay or to hold ejaculation because dapoxetine is doing it for you. Dapoxetine will delay/improve your IELT within 3 months from the first assumption". Instructions were given to each new patient enrolled in the study, and dapoxetine was administered for six m,onths. The first author gave the first set of instructions to all new patients enrolled from January 2nd 2015 to July 2nd 2015, and gave the second set of instructions to all patients enrolled from July 3rd 2015 to January 2nd 2016. The second Author gave the second set of instructions from January 2nd 2015 to July 2nd 2015 and gave the first set of instructions from July 3rd 2015 to January 2nd 2016. The patients who received set 1 made up Group 1, the patients who received set 2 made up Group 2.

The following variables were assessed before and during the course of dapoxetine assumption: stopwatch-measured average IELT; the clinical global impression of change (CGIC) in PE. The IELT was defined as the time elapsed between penetration and ejaculation; an ejaculation which occurred before penetration was assigned an IELT of 0 min. The mean IELT is intended an entire one-month measurement after 3 months of use and was measured with a partner-operated stopwatch. The CGIC is a subjective answer to the following question: 'Compared to the start of the study, would you describe your premature ejaculation problem as much worse, worse, slightly worse, no change, slightly better, better or much better?' (7). The side effects of dapoxetine were recorded as well. The differences in side effects and the differences between the number of patients studied in each center and belonging to Group 1 or Group 2 were assessed using the chi^2 test; the differences between paired data were assessed using the Wilcoxon test while the differences between the independent data were assessed using the Mann-Whitney test. The levels of significance maintained an overall P value of 0.05 and were calculated according to the O'Brien-Fleming stopping boundary for endpoints (9, 10).

III. Results

One hundred and eighty-one people were evaluated for potential enrollment; 21 did not satisfy the inclusion criteria. Thirty-six were lost to follow up due to insufficient therapeutic effect (25 cases), side effects (6 cases) and protocol violations (incorrect assumption of dapoxetine) (5 cases). One hundred and twenty-four patients were studied.

The clinical and demographic data of each population studied are presented in Table 1. No significant differences emerged between Group 1 and Group 2 patients.

The stopwatch-measured average IELT, the CGIC and the side effects in Groups 1 and 2 are reported in Table 2. There were no significant differences among the baseline values of IELT and PEP which, however, significantly improved after dapoxetine assumption; Group 2 patients achieved a greater improvement in IELT and CGIC than Group 1. No significant differences occurred between the data obtained in Piacenza and the data obtained in Ferrara. The side effects were similar in both groups.

IV. Discussion and Conclusions

Our data showed that medical instructions influence dapoxetine efficacy for lifelong PE. The fact that operator-dependent variability in terms of efficacy is mundane confirms that the physician's instructions are critical for dapoxetine efficacy. It is likely that no operator-dependent variability occurred since the researchers have approximately thirty years of experience in the field, and they are truly skilled in giving instructions as to the assumption of medications.

From a psychological point of view, lifelong PE can be regarded as anxiety which manifests itself as a sexual dysfunction (11). Thus, we sought to develop a set of instruction with the aim of overcoming the anxiety of the patients. We decided a prior to develop (at least in part) a set of instructions for Group 2 based on the instructions given in the course of integrated task couple therapy for psychogenic sexual dysfunctions where the technique used by the therapist to instruct the couple regarding sexual homework is critical for success (12). The physician's tone was compelling and was used to

stress the efficacy of the drug and the power of the medical prescription (12). The patients were instructed not to try to delay or to hold back ejaculation (a psychological tactic regarded as stressing (11); other physicians indicated that the time required to improve their ejaculation was long in order to avoid any expectancy (and consequent anxiety) on the part of the patient regarding the interval between the first assumption of the drug and its taking effect. To further overcome patient anxiety as much as possible, we chose to carry out a fixed dose study using the highest available dapoxetine dosage (60 mg) in order to be able to achieve the highest drug efficacy and to reassure the patients regarding dapoxetine efficacy as soon as possible.

The patients belonging to the first group received dapoxetine without any explanation regarding drug activity and the interval between the first assumption and when the drug takes effect; thus, it may be presumed that their anxiety regarding when and if dapoxetine functions may still have been present.

The Authors felt that it was not possible to take into account all the other variables involved in the patient-physician relationship and sexual and other relationships of a couple; thus a multicenter cross-over study was adopted. The fact that no operator dependent variability could be found indicated that our data could be regarded as reliable.

This paper has some limitations, because it is very difficult to produce perfectly balanced results when the subjects of study are men with an anxiety-based disease such as PE. In this regard, we chose to examine only couples who cohabitated > 1 year to avoid any differences regardingt marital status as much as possible. The first potential limitation of this study was that the Authors could only presume that the instructions for Group 2 were more soothing than those for Group 1 since direct proof of the influence of the physicians' instructions regarding the anxiety of the patients could not be achieved. In any case, a study in which two different sets of instructions were administered to the same group of patients is confounding. Furthermore, any medical instructions for the use of a drug which might resolve a lifelong disease could be perceived by the patient as soothing "per se" (13); however, the more detailed the instructions, the more soothing they are (14). The instructions for Group 2 were more detailed than those for Group 1; however, in the literature, we could not find a statement that soothing and detailed instructions might improve the efficacy of a drug, even for a psychogenic disease. In this regard, this paper might represent a true novelty. A second limitation of the present study was the absence of a placebo branch; however, a preliminary attempt to use a placebo induced the vast majority of the patients (80%) to refuse to participate in the present study, further a number of placebo controlled studies were conducted using Dapoxetine, which showed to be more efficient for resolving lifelong PE than placebo (15) . When the patients were asked the reason for their refusal, they all answered that they were impatient to resolve their symptoms. Impatience is actually a characteristic of PE patients (11). Thus the Authors were compelled not to use a placebo branch because this percentage of refusals to participate would induce sampling errors (9). A third limitation was that it was very difficult to fully evaluate the distress, annoyance or interpersonal difficulty of each patient before and after dapoxetine assumption, mainly in the absence of a validated questionnaire (in fact, the premature ejaculation profile (PEP), used to evaluate ejaculatory capacity and satisfaction with the treatment, could not be used because it has not been translated into Italian and validated in Italy (16). In any case, it ws thought that stopwatch-measured average IELT and the CGIC might be sufficient for evaluating results.

Although the characteristics of premature ejaculation have been established, the exact etiology is largely unknown. Genetic, neurobiological, pharmacological, psychological, urological and endocrine factors have all been proposed. In lifelong PE, there are convincing data to support roles for genetic and psychological factors, either causal, or secondary to PE for the latter (17); however, our data seem to support the hypothesis that lifelong PE could depend, at least in part, on psychological factors.

Genetic polymorphisms located on the SLC6A4 gene codifying for the 5-HT transporter (5-HTT), the major regulator of serotonic neurotransmission, have been linked to the pathogenesis and risk of PE. A recent meta-analysis has shown that the *5-HTTLPR* gene polymorphism was associated with a significantly decreased risk for lifelong PE risk in Caucasians (18). As a matter of fact, the 5-HTTLPR gene is associated with prosocial behavior due to its effects on anxiety in social situations (19). Thus, even in the case that lifelong PE has a genetic etiology, it is not surprising that medical instructions of a soothing nature improve dapoxetine efficacy, because of the effects on anxiety of the gene involved.

In conclusion, since the main cause of dropping out of the study was the poor efficacy of dapoxetine in improving ejaculation (six months after the beginning of the study, approximately 38% of the patients autonomously stopped dapoxetine assumption (20), it is likely that soothing and/or detailed instructions for taking the drug could improve patient compliance to dapoxetine treatment.

List of abbreviations

Premature ejaculation = PE;
Intravaginal ejaculatory latency time = IELT;
Clinical global impression of change = CGIC;
Premature ejaculation profile = PEP.

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Table 1: Clinical and demographical data of the population studied. Data are presented as medians and as ranges (min-max). Group 1 was made up of patients who received dapoxetine 60 mg with the following instructions: "Take one tablet two hours before sexual intercourse; dapoxetine will delay your IELT beginning with the first sexual intercourse". Group 2 was made up of patients the second was: "You will assume one tablet two hours before sexual intercourse; do not try to delay or to hold back ejaculation because dapoxetine is doing it for you. Dapoxetine will delay/improve your intravaginal ejaculatory latency time (IELT) within 3 months from the first assumption".

			Patients examined in Ferrara	Patients examined in Piacenza
Group 1	Number		32	28
	Age (in years)		28 (21-34)	29 (21-36)
	Age at first sexual intercourse (in years)		18 (16-20)	17 (14-20)
	Duration of premature ejaculation (in years)		10 (7-13)	11 (9-13)
	Social status	Workmen	11	12
		Artisans	5	3
		Employees	4	3
		Graduates	6	5
		Businessmen	2	1
		Undergraduates	4	4
Group 2	Number		28	36
	Age (in years)		29 (21-36)	29 (21-36)
	Age at first sexual intercourse (in years)		17 (14-20)	18 (16-20)

	Duration of premature ejacula		12 (9-15)	12 (10-15)
	Social status	Workmen	10	12
		Artisans	4	6
		Employees	4	5
		Graduates	5	6
		Businessmen	1	2
		Undergraduat	4	5

Table 2: Results of a prospective multicenter study where the patients were all instructed to take dapoxetine 60 mg, one tablet, two hours before sexual intercourse. Data are presented as medians and as ranges (min-max). Two different sets of instructions were given to the patients. The first set of instructions was given to Group 1 : "Take one tablet two hours before sexual intercourse; dapoxetine will delay your ejaculation beginning with the first sexual intercourse"; the second set of instructions was given to Group 2: "Take one tablet two hours before sexual intercourse; do not try to delay or to hold back ejaculation because dapoxetine is is doing it for you. Dapoxetine will delay/improve your intravaginal ejaculatory latency time (IELT) within 3 months from the first assumption".

Key: * headache; **one case of headache and one case of nausea; ***one case of nervousness + nausea, one case of headache and one case of nausea; ****one case of nervousness + headache + nausea, one case of headache + nausea, vs. = versus.

Comparisons:

a1 vs. a2, a3 vs. a4, a1 vs. a3, a2 vs. a4, b1 vs. b2, b3 vs. b4, c1vs. c2, c3 vs. c4, c1 vs. c3, c2 vs. c4, d1 vs. d2, e1 vs. e2, e2 vs. e3, f1 vs. f2, f3 vs. f4, f1 vs. f3, and f2 vs. f4: p not significant.
a1 vs. b1, a2 vs. b2, a3 vs. b3, a4 vs. b4, c1 vs. d1, c2 vs. d2, c3 vs. d3, d4 vs. d4: $p<0.01$.
b1 vs. b3, b2 vs. b4, d1 vs. d3, d2 vs. d4, e1 vs. e3, e2 vs. e 4: $p<0.01$.

	Group 1					
	Stopwatch-measured average intravaginal ejaculatory latency time (IELT) in minutes		Premature Ejaculation Profile (PEP) score		Number (percentage) of patients who described their premature ejaculation as slightly better, better, or much better after dapoxetine assumption (e)	Number (percentage) of patients who suffered from side effects (f)
	Before dapoxetine assumption (a)	After dapoxetine assumption (b)	Before dapoxetine assumption (c)	After dapoxetine assumption (d)		
Patients examined in Ferrara(1)	0.8 (0.5 -1.1)	2.6 (0.8-3.2)	15 (11-19)	10 (5-17)	17 (53.1%)	1* (3.1%)
Patients examined in Piacenza(2)	0.8 (0-1)	2.5 (0.9-3.1)	16 (11-19)	11 (6-16)	15 (53.6%)	2** (7.1%)
Group 2						
Patients examined in Ferrara(3)	0.8 (0.2-1)	5.4 (1.7-9.8)	16 (11-20)	7 (2-13)	22 (78.6%)	2*** (7.1%)
Patients examined in Piacenza (4)	0.8 (0.1± 1.1)	6.0 (1.8-10.0)	16 (11-20)	7 (3-14)	30 (83.3%)	2**** (5.6%)

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 17 Issue 1 Version 1.0 Year 2017
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Anti-hyperglycemic and in vivo Antioxidant Activities of Aqueous Extract of *Blighiasapida* Stem Bark in Alloxan-Induced Diabetic Rats

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Abstract- *Blighiasapida* is a plant belonging to the family of Sapindaceae. This study is aimed at evaluating the hypoglycemic and possible in vivo antioxidant activities of the aqueous extract of the stem bark of the plant for 21 days in alloxan-induced diabetic rats. Administration of the extract at 100mg/kg body weight significantly ($P<0.05$) decreased blood glucose levels, increased body weight as well as increased the activities of antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase in the plasma and liver tissues of diabetic rats. Also, the concentration of reduced glutathione increased in the plasma and liver tissues of the diabetic rats while the levels of malondialdehyde and protein carbonyl significantly decreased in the plasma and liver tissues of alloxan-induced diabetic rats during the course of the experiment. These are indications of antihyperglycemic and antioxidant properties of the stem bark of Blighiasapida with 100mg/kg body weight of the extract showing good hypoglycemic and antioxidant activities by comparing favourably well with metformin, a standard hypoglycemic drug.

Keywords: *blighiasapida, diabetes, antioxidant enzymes, epidemic, biomolecules.*

GJMR-B Classification: *NLMC Code: QV 55*

Strictly as per the compliance and regulations of:

Anti-hyperglycemic and *in vivo* Antioxidant Activities of Aqueous Extract of *Blighiasapida* Stem Bark in Alloxan-Induced Diabetic Rats

Amira, Philip O [α] & Oloyede, Hussein O. B [σ]

Abstract- ***Blighiasapida*** **is a plant belonging to the family of Sapindaceae. This study is aimed at evaluating the hypoglycemic and possible *in vivo* antioxidant activities of the aqueous extract of the stem bark of the plant for 21 days in alloxan-induced diabetic rats. Administration of the extract at 100mg/kg body weight significantly (P<0.05) decreased blood glucose levels, increased body weight as well as increased the activities of antioxidant enzymes catalase, glutathione peroxidase and superoxide dismutase in the plasma and liver tissues of diabetic rats. Also, the concentration of reduced glutathione increased in the plasma and liver tissues of the diabetic rats while the levels of malondialdehyde and protein carbonyl significantly decreased in the plasma and liver tissues of alloxan-induced diabetic rats during the course of the experiment. These are indications of antihyperglycemic and antioxidant properties of the stem bark of *Blighiasapida* with 100mg/kg body weight of the extract showing good hypoglycemic and antioxidant activities by comparing favourably well with metformin, a standard hypoglycemic drug.**

Keywords: blighiasapida, diabetes, antioxidant enzymes, epidemic, biomolecules.

I. Introduction

Diabetes mellitus is a multifactorial disease, which is characterized by hyperglycemia (Ugochukwu *et al.*, 2003), lipoprotein abnormalities (Scoppola *et al.*, 2001), raised basal metabolic rates (Okwu *et al.*, 2006), defect in reactive oxygen species scavenging enzymes and altered intermediary metabolism of major food substances (Unwin *et al.*, 2001). Diabetes being a major degenerative disease is found in all parts of the world and it is becoming the third most lethal disease of mankind and rapidly increasing. It is affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders (Saidu *et al.*, 2012).

Diabetes mellitus is a group of metabolic disease caused by a defect in insulin production, insulin action or both.

Type 1 diabetes is caused by a lack of insulin due to the destruction of insulin-producing β – cells in the pancreas. Type 2 diabetes, the most common form of diabetes is caused by a combination of factors, including insulin resistance, a condition in which the body's muscle, fat and liver cells do not use insulin effectively.

Too much glucose circulating in the blood results in hyperglycemia, one of the major symptoms of diabetes. Hyperglycemia causes many of the health problem associated with diabetes, including eye, kidney, heart disease and nerve conditions.

The World Health Organization (WHO) in its 2014 release repoeted that the prevalence of diabetes has reached epidemic proportions. In 2014 the global prevalence of diabetes was estimated to be 9% among adults aged 18+ years. In 2012, an estimated 1.5 million deaths were directly caused by diabetes. More than 80% of diabetes deaths occur in low- and middle-income countries (WHO, 2014).

Diabetes mellitus is associated with an increase in reactive oxygen species (ROS) generation by mononuclear cells and an increased oxidative load resulting in oxidative damage to lipids, proteins and DNA (Marfella *et al.*, 1995; Giugliano *et al.*, 1997; Paoliso and Giugliano, 1996).

Chronic hyperglycemia and subsequent augmentation of reactive oxygen species (ROS) deteriorate β-cell functions and increase insulin resistance which leads to the aggravation of type 2 diabetes. In addition, chronic hyperglycemia and ROS are also involved in the development of atherosclerosis which is often observed under diabetic conditions (Kaneto *et al.*, 2010).

It has been shown that ROS are produced in various tissues under diabetic conditions (Baynes and Thorpe, 1999). There are several sources, of ROS in cells such as the nonenzymatic glycosylation reaction, the electron transport chain in mitochondria, and membrane-bound NADPH oxidase (Brownlee, 2001; Harrison *et al,* 2003; Mohazzab *et al*, 1994).

Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion. This process is called β-cell glucose toxicity which is often observed under diabetic conditions (Evans *et al*, 2003).It is also known that lipotoxicity is also involved in the deterioration of β-cell function found in type 2 diabetes (Kaneto *et al*, 2010).

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Blighiasapida is a plant belonging to the family of Sapindaceae. It is commonly known as ackee. In Nigeria, it is called Gwanja Kusa (Hausa), Isin (Yoruba) and Okpu (Igbo). It is an evergreen tree of about 33 to 40ft (10-12m) with a dense crown of spreading branches. The leaves are compound with three to five pairs of oblong, ovate-oblong, or elliptical leaflets 1.5-3.0cm long. The seed of the fruit is not edible, whereas the fleshy aril is edible. The fruit is known to contain saponins, which are hemolytic (Aderinola *et al.*, 2007).

Most of the earlier studies on *Blighiasapida* have been on the nutritional qualities of the root (Abolaji *et al*, 2007) and the leaves as a dry season feed resource for West African dwarf goats in the Northern savanna zone of Nigeria (Aderinola *et al*, 2007). The repellant potential of the fruit part components against stored-product insect pests (Khan and Gumbs, 2003) as well as neutropenia and thrombocytopenia effects of the aqueous and lipid extracts of the unripe fruit have been investigated in mice (Gardiner *et al*, 1996). More recently, the physicochemical properties of the oil from the fruit of the species and toxicological evaluation of the oil – based diet in Wister rats have been investigated (Oladiji *et al*, 2009).

However, the scanty information on the antioxidant activity of extract of *Blighiasapida* stem bark and its anti-hyperglycemic effect prompted this study.

Tree bark is an important component of African traditional medicine as herbal medicine is still the main source of health care for the majority of Africans and in particular, Nigerians.

There has been increasing demand for the use of plant products with anti-diabetic activity. The prohibitively high cost, unavailability, uncertainty of use of the common anti-diabetic agents during pregnancy and undesirable side effects of these drugs have been some of the factors limiting their use and leading to a preference for anti-diabetic drugs of plants origin. This study is thus aimed at isolation of hypoglycemic agents from readily available *Blighiasapida*.

II. Materials and Methods

Chemicals: All chemicals used were of analytical grade and items are products of BDH and Sigma Chemical Ltd., UK and Accu-chek ® Advantage, Roche Diagnostic, Germany.

Animals: Male albino rats (*Ratusnorvegicus*) weighing between 100g and 120g were used for the experiment. The rats were bred in the animal holding of the Department of Anatomy and Cell Biology, Obafemi Awolowo University, Ile-Ife and were maintained on standard rat pellets (Ladokun feeds, Ibadan, Nigeria), and were given water *ad libitum*.

Sourcing for the Tree Bark of Blighiasapida: A sizeable quantity of the tree bark of *Blighiasapida* was obtained from the compound of the Federal Polytechnic, Ado Ekiti, Nigeria.

Identification of Plant: The fruits and leaves of *Blighiasapida* plant were obtained from the compound of the Federal polytechnic, Ado Ekiti, Ekiti State, Nigeria and were used for the purpose of authentication of the identity of the plant at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The voucher number of identification is UIH624.

Processing of sample and preparation of extract: The sample obtained was air-dried at room temperature for fifty-six(56) days until a constant weight was obtained. The air-dried tree bark of *Blighiasapida* was pulverized. 100g of the pulverized sample was extracted with 800ml of distilled water for seventy-two (72) hours in an extractor. The aqueous extract was obtained by filtering with What man filter paper and subsequently freeze-dried in Armfield freeze-drier for ten (10) days.

Induction of experimental diabetes mellitus: After an overnight fasting, rats were induced by intraperitoneal administration of alloxan monohydrate at a dose of 120mg/kg body weight. Alloxan monohydrate was freshly dissolved in distilled water and maintained on ice prior to use. Four days after the administration, the animals were fasted for 16 hours and blood glucose levels were determined in mg/dl using a digital glucometer (Accu-chek ®, advantage, Roche, Diagnostic, Germany) and animals which had basal glycemia levels of 125mg/dl were used in the experiment.

Experimental Design: Randomized Complete Block Design (RCBD) method was used.

Eighty male albino rats were grouped as follows:

Group 1: Control group administered with distilled water orally.

Group 2: The alloxan-induced diabetic group left untreated

Group 3: The alloxan-induced diabetic group treated with oral administration of distilled water extract of *Blighiasapida* at 100mg/1000g body weight

Group 4: The alloxan-induced diabetic group treated with oral administration of Metformin hydrochloride at 21.4mg/1000g body weight.

All the animals were fed with vital finisher made up of maize and soya bean mainly. The administration of the extracts as written above was carried out every 24 hours for 21 days.

Analysis of the various parameters stated was carried out weekly after diabetes detection, for three weeks.

Repeated administration of the aqueous extract of Blighiasapidastem bark in control and diabetic groups: The fasting blood glucose levels of all groups were measured and then the extract dissolved in distilled

water. The solution of the extract was administered to one of the diabetic groups orally at 100mg/kg body weight once a day for twenty-one (21) days.The diabetic control and untreated (without alloxan induction). Body weight and blood glucose levels of the groups were monitored daily, blood sample was obtained from the tail vein of the animals and their fasting blood glucose level was determined in mg/dl using a digital glucometer (Accu-chek ® Advantage, Roche Diagnostic, Germany). Five animals each were sacrificed from each of the four groups by chloroform anaesthesia and the blood and liver obtained from them. The plasma was obtained from the blood by using centrifuge at 3000g for 15 minutes. The plasma and liver so obtained were stored in phosphate buffer (0.1M, pH = 7.0) maintained below -20^{0}C until required for analysis.

In vivoantioxidant assay: Liver tissues were homogenized with cold 1.5% KCl to make a 10% homogenate.

Determination of the activity of Catalase (CAT): Catalase activity was determined in the lysate using Aebi's method (Aebi, 1984).

Determination of the activity of Superoxide dismutase (SOD): This method is well described by Mccord and Fridovich (1969).

Determination of the activity of Glutathione Peroxidase (GPx): Glutathione peroxidase (GPx) was measured by the method described by Rotruck *et al.* (1973).

Determination of reduced glutathione (GSH): Reduced glutathione (GSH) was measured by the method of Beatler*et al.* (1963).

Determination of Malondialdehyde (MDA): Total amount of lipid peroxidation products present in the samples was estimated by the thiobarbituric acid (TBA) method which measures the malondialdehyde (MDA) reactive products according to the method of Ohkawa *et al.*, (1979).

Determination of Protein Carbonyl Content: The protein carbonyl content was assayed according to a previous method of Levine *et al* (1990).

Determination of Protein: Protein determination was carried out according to the method of Lowry *et al.,* (1951) as described by Holme and Peck, (1998).

Statistical Analysis: Data were expressed as mean ± S.E.M. of five replicates and subjected to one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to determine significant differences in all the parameters. Values were considered statistically significant at $P<0.05$.

III. Results

Blood Glucose Level: The administration of aqueous extract of *Blighiasapida* stem bark was found to significantly ($P<0.05$) reduce the blood glucose in diabetic albino rats at the end of the experiment (Table 1). The effect was more rapid in the first week of administration and compared favourably well with metformin-treated diabetic rats.

Weight gain or loss: There was a significant reduction ($P<0.05$) in the weight gained by the untreated diabetic rats when compared with the metformin-treated and extract-treated groups. Generally, the effect of treatment with 100mg/kg body weight of aqueous extract of *B. sapida* stem bark compared favourably well with that of metformin hydrochloride which is a known standard drug for diabetes.

Catalase activity: Specific activity of the antioxidant enzyme catalase was found to be increased ($P<0.05$) plasma and liver following administration of aqueous extract of *B. sapida* stem bark while the administration of metformin, a standard antidiabetic drug did not seem to have any ameliorative effect on the reduced specific activity of catalase in the plasma and liver of diabetic rats when compared with the untreated diabetic rats (Table 3).

Glutathione peroxidase (GPx) activities: A significant increase ($P<0.05$) was noticed in the specific activity of glutathione peroxide in the plasma of diabetic rat following administration of aqueous extract of *B. sapida* stem bark at the later stage of the experiment. On the other hand, the specific activity of glutathione peroxidase in the liver of diabetic rats did not increase but significantly reduced ($P<0.05$) during the course of the experiment, a result similar to the one obtained for the untreated diabetic rats (Table 4).

Table 1: Blood Glucose level of alloxan-induced diabetic rats following administration of Aqueous extract of *Blighiasapida* stem bark.

Groups	Serum Glucose Level (mg/dl)			
	0 day	7th day	14th day	21st day
Untreated control	91.50 ± 1.94^a	90.41 ± 2.50^a	90.21± 1.61^a	88.10 ± 2.02^a
Diabetic control	154.80 ± 14.00^b	172.41± 17.32^b	203.50 ±11.20^b	253.00 ± 13.20^b
Diabetic + Aqueous extract	154.80 ± 14.00^b	86.40±5.43^a	74.00±4.48^c	58.00 ± 6.04^c
Diabetic + Metformin	153.84 ±10.26^b	113.75 ±5.41^c	70.75 ± 6.50^c	57.00 ± 9.60^c

Values are mean of five determinations ±S.E.M. Values with different superscript in the row and column differ significantly ($p<0.05$)

Table 2: Body weight of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida* stem bark.

Groups	Average body weight of animals (g)			
	0 day	7th day	14th day	21st day
Untreated control	129.20 ±2.30[a]	133.70 ±1.09[a]	146.20± 1.12[a]	157.00± 1.16[a]
Diabetic control	132.01 ± 1.09[a]	125.20 ±2.01[b]	112.00 ±0.98[b]	98.20 ±2.02[b]
Diabetic + Aqueous extract	131.00 ±6.06[a]	149.00 ±7.12[c]	157. 00 ±6.57[c]	124.00 ±6.06[c]
Diabetic + Metformin	132.00 ± 2.96[a]	116.25 ± 5.41[b]	127.50 ± 6.50[d]	147.50 ±9.60[d]

Values are mean of five determinations ± S.E.M. Values with different superscript in the row and column differ significantly ($p<0.05$)

Table 3: Specific activity of catalase in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida* stem bark

Tissue	Group of animal	Specific activity of catalase (Units/mg protein) (x10^{-2})			
		0 day	7th day	14th day	21st day
Plasma	Untreated control	5.86± 0.14[a]	5.91± 0.20[a]	5.86± 0.16[a]	5.82± 0.16[a]
	Diabetic control	3.86± 0.70[b]	3.52± 0.20[b]	2.14±0.10[b]	1.27± 0.80[b]
	Diabetic + Aqueous extract	3.86± 0.70[b]	4.25± 0.90[c]	7.81± 1.80[a]	15.66± 3.20[c]
	Diabetic + Metformin	5.30± 0.28[a,a]	6.10± 0.10[a]	2.10± 0.07[b]	1.90± 0.62[b]
Liver	Untreated control	178.48± 4.90[a]	179.32± 3.20[a]	178.52± 5.20[a]	176.05± 5.10[a]
	Diabetic control	92.26± 6.30[b]	92.52± 2. 10[b]	79.16± 5.20[b]	49.56± 6.20[b]
	Diabetic + Aqueous extract	97.26± 6.30[b]	195.02± 3.93[c]	289.36± 8.30[c]	190.85± 3.20[a]
	Diabetic + Metformin	97.30± 0.28[b]	84.30± 0.28[b]	87.00± 0.31[b]	65.80± 0.63[c]

Values are mean of five determinations ± S.E.M. Values with different superscript in the row and column differ significantly ($p<0.05$)

Table 4: Specific activity of Glutathione peroxidase (GPx) in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida* stem bark

Tissue	Group of animal	Specific activity of Glutathione peroxidase (Units/mg protein) (XI0·[5])			
		0 day	7th day	14th day	21st day
Plasma	Untreated control	13.68± 0.48[a]	13.54± 0.39[a]	13.67± 0.46[a]	13.83± 0.49[a]
	Diabetic control	13.61± 0.18[a]	11.3S± 0.15[a]	10.23± 0.18[b]	8.26± 0.16[b]
	Diabetic + Aqueous extract	13.61± 0.18[a]	7.96± 0.61[b]	6.10± 0.08[c]	8.85± 0.85[b]
	Diabetic + Metformin	I3.50± 0.6I[a]	4.94± 0.53[c]	4.79± 0.59[c]	6.10± 0.4I[c]
Liver	Untreated control	47.00± 1.02[a]	47.00±1.I2[a]	48.00± 1. 02[a]	46.00± 1.01[a]
	Diabetic control	30.40± 0.72[b]	25.10± 0.69[b]	I7.30± 0.59[b]	10.20± 0.30[b]
	Diabetic + Aqueous extract	30.40± 0.72[b]	44.I0± 0.99[a]	32.70± 0.40[c]	18.00± 0.31[c]
	Diabetic + Metformin	3I.60± 0.53[b]	49.00± 0.53[a]	4780± 0.40[a]	60.00± 0.35[a]

Values are mean of five determinations ± S.E.M. Values with different superscript in the row and column differ significantly ($p<0.05$)

Superoxide dismutase (SOD) activity: A significant increase ($P<0.05$) in the specific activity of superoxide dismutase was observed in the plasma and liver diabetic rats administered with aqueous extract of *B. sapida* stem bark similar to what was observed in those treated with metformin, a standard antidiabetic drug. However, the specific activity of superoxide dismutase in the plasma and liver of untreated diabetic rats was found to reduce significantly ($P<0.05$) during the course of the experiment (Table 5).

Reduced glutathione: Table 6 shows the effect of administration of aqueous extract of *B. sapida* stem bark on concentration of reduced glutathione (GSH) in plasma and liver of diabetic rats. A significant increase ($P<0.05$) in the oxidant was noticed in the plasma and

liver of diabetic rats following the administration of aqueous extract of *B. sapida* stem bark.

Malondialdehyde: A significant reduction (P<0.05) in the level of malondialdehyde (MDA) was observed in the plasma and liver of diabetic rats following the administration of aqueous extract of *B. sapida* stem bark (Table 7). On the other hand changes in the concentration of malondialdehyde in plasma and liver tissues of diabetic rats did not follow a particular pattern following the treatment of diabetic rats with metformin hydrochloride, a standard antidiabetic drug.

Protein carbonyl: A significant reduction (P<0.05) was noticed towards the end of the experiment after an initial increase in the concentration of protein carbonyl in the plasma and liver tissues of diabetic rats following the administration of aqueous extracts of *B. sapida* stem bark (Table 8). A similar result was obtained for the diabetic rats treated with standard antidiabetic drug, metformin.

Table 5: Specific activity of superoxide dismutase (SOD) in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida*stem bark

Tissue	Group of animal	Specific activity of superoxide dismutase (SOD (Units/mg protein) ($x10^{-3}$)			
		0 day	7thday	14th day	21stday
Plasma	Untreated control	10.88± 1.23[a]	19.53± 1.06[a]	22.11± 1.00[a]	19.93± 1.23[a]
	Diabetic control	7.91± 1.40[b]	5.25± 1.30[b]	3.28± 1.0l[b]	1.96± 0.91[b]
	Diabetic + Aqueous extract	7.91± 1.40[b]	27.12± 4.05[c]	24.03± 3.56[a]	28.64± 7: 12[c]
	Diabetic + Metformin	8.10± 1.10[b]	12.90± 0.70[d]	17.20± 0.30[c]	28.70± 0.30[c],
Liver	Untreated control	55.87± 9.65[a]	55.87± 9.65[a]	66.01± 7.51[a]	51. 78± 1.20[a]
	Diabetic control	35.40± 5.98[b]	19.87± 4.43[b]	11.35± 3.25[b]	9.36± 1.54[b]
	Diabetic + Aqueous extract	35.40± 5.98[b]	150.52± 1.93[c]	161.80± 2.27[c]	1153.95± 12.11[c]
	Diabetic + Metformin	58.70± 1.10[b]	12.90± 0.70[b]	17.20± 0.30[d]	28.70± 0.30[d].

Values are mean of five determinations ± S.E.M. Values with different superscript in the row and column differ significantly (p<0.05)

Table 6: Concentration of reduced glutathione (GSH) in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida* stem bark.

Tissue	Group of animal	Concentration of Glutathione (GSH) (mM/mg tissue)			
		0 day	7th day	14th day	21st day
Plasma	Untreated control	3.63± 0.56[a]	3.45± 0.32[a]	3.63± 0.56[a]	3.65± 0.56[a]
	Diabetic control	3.21± 0.43[a]	2.51± 0.55[b]	2.11± 0.30[b]	1.82± 0.25[b]
	Diabetic + Aqueous extract	3.21± 0.43[a]	2.83± 0.34[b]	2.63± 0.41[c]	3.15± 0.25[c]
	Diabetic + Metformin	3.64± 0.15[a]	4.71± 0.l5[c]	l.86± 0.18[b]	4.27± 0.99[a]
Liver	Untreated control	1.21± 0.19[a]	1.21± 0,.19[a]	1.56± 0.15[a]	1.35± 0.20[a]
	Diabetic control	2.54± 0.53[b]	1.83± 0.32[b]	1.24± 0.22[b]	1.03± 0.16[b]
	Diabetic + Aqueous extract	2.54± 0.53[b]	1.24± 01.14[a]	1.46± 0.10[b]	4.06± 0.27[c]
	Diabetic + Metformin	2.35± 0.03[b]	1.18± 0.03[a]	1.92± 0.18[c]	1.93± 0.07[a]

Values are mean of five determinations ± S.E.M. Values with different superscript in the row and column differ significantly (p<0.05)

Table 7: Concentration of malondialdehyde (MDA) in plasma and liver of alloxan-induced diabetic albino rats following administration of Aqueous extract of *Blighiasapida stem* bark

		Concentration of malondialdehyde (MDA) (mmol/mg tissue) (xl0·^{5})			
Tissue	Group of animal	0 day	7thday	14thday	21stday
Plasma	Untreated control	1316.99± 0.16^{a}	1428.60± 0.13^{a}	1316.90± 0.16^{a}	1439.80± 0.12^{a}
	Diabetic control	1619.69± 0.08^{a}	2002.10± 0.07^{b}	4698.70± 0.09^{b}	7023.60± 0.06^{b}
	Diabetic + Aqueous extract	1619.69± 0.08^{a}	1372.55± 0.05^{a}	1065.36± 0.06^{a}	1125.82± 0.04^{a}
	Diabetic + Metformin	280.00±0.11^{b}	420.00± 0.11^{c}	380.00± 0.51^{d}	500.00± 0.17^{c}
Liver	Untreated control	1286.70± 0.01^{a}	l286.70± 0.01^{a}	1096.40± 0.01^{a}	1193.00± 0.01^{a}
	Diabetic control	1513.10± 0.08^{b}	1735.20± 0.08^{b}	2012.30± 0.08^{b}	2523.20± 0.06^{b}
	Diabetic + Aqueous extract	1513. 10± 0.08^{b}	1388.89± 0.06^{a}	1197.71± 0.06^{a}	1040.S5± 0.05^{a}
	Diabetic + Metformin	1720.00± 0.08^{b}	2000.00± 0.07^{b}	1900.00± 0.08^{b}	3200.00± 0.07^{d}

Values are mean of five determinations ± S.E.M. Values with different superscript in the row and column differ significantly ($p<0.05$)

Table 8: Concentration of protein carbonyl in plasma and liver of alloxan-induced diabetic albino rats administration of Aqueous extract of *Blighiasapida* stem bark

		Concentration of protein carbonyl (micromol carbonyl/mg tissue)			
Tissue	Group of animal	0 day	7th day	14th day	21st day
Plasma	Untreated control	0.56± 1.05E-05^{a}	0.52± 1.12 E-05^{a}	0.59± 1.05E-05^{a}	0.60± 1.03E-05^{a}
	Diabetic control	0.46± 6.66E-07^{b}	0.68± 8.63 E.07^{b}	1.26± 7.65E-07^{b}	1.80± 8.20E-07^{b}
	Diabetic + Aqueous extract	0.46± 6.66E-07^{b}	1. 09± 1. 11 E-06^{c}	0.98± 1.15E-06^{c}	0.32± 9.09E-07^{c}
	Diabetic + Metformin	0.53± 1.36E-05^{a}	0.55± 1.36 E-05^{a}	0.74± 1.81E-05^{a}	0.53± 1.39E-05^{a}
Liver	Untreated control	2.53± 1.08E-05^{a}	2.53± 1.09 E-05^{a}	2.47± 1.11E-05^{a}	2.51± 1.08E-05^{a}
	Diabetic control	0.78± 7.80E-07^{b}	1.86± 8.22 E-07^{b}	2.60± 6.11E-07^{b}	3.82± 8.52E-07^{b}
	Diabetic + Aqueous extract	0.78± 7.80E-07^{b}	2.08± 1.05E-06^{c}	0.94± 1.05E-06^{c}	0.66± 7.42E-07^{c}
	Diabetic + Metformin	1.27± 6.22E-06^{c}	2.06± 6.22E-06^{c}	1.97± 2.71E-05^{a}	2.08± 5.I2E-06^{d}

Values are mean of five determinations ± S.E.M. Values with different superscript in the row and column differ significantly ($p<0.05$)

IV. Discussion

The increase in blood glucose concentration is an important characteristic feature of diabetes. *Blighiasapida* extract produced significant hypoglycemic effect on diabetic rats, and by day 14, the glucose levels tended towards normalcy as found in the control rats. Phytochemical screening of the aqueous extract of the root bark of *B. sapida* had indicated the presence of saponins (Saidu*et al.*, 2012), which have been reported to possess hypoglycemic activity in diabetic rabbits (Abdel-Hassan *et al.*, 2000).

The marked increase in the body weight in the *B. sapida* stem bark extract-treated rats could be attributed to the increase in the metabolic activity of their body systems. This clearly indicates that the plant extract increase glucose metabolism which enhanced body weight gain in rats. This observation was reported by Sunmonu and Afolayan (2013). According to these authors, *Artemisia afra* leaves and stem increased the body weight of diabetic rats. It is interesting to note that the effect of *B. sapida* stem bark aqueous extract at the dose of 100mg/kg body weight compared favorably well with metformin.

Diabetes mellitus is associated with an increase in reactive oxygen species (ROS) generation by mononuclear cells and an increased oxidative load resulting in oxidative damage to lipids, proteins and DNA. Acute hyperglycemia has been shown to result in an increase in blood pressure, which is prevented by antioxidants; this suggests that acute hyperglycemia probably causes increased generation of ROS.

Chronic hyperglycemia and subsequent augmentation of reactive oxygen species (ROS) deteriorate β – cell functions and increase insulin resistance which leads to the aggravation of type 2 diabetes (Kaneto*et al.*, 2010).

It has been shown that ROS are produced in various tissues under diabetic conditions (Baynes and Thorpe, 1999). There are several sources of ROS in cells

such as the nonenzymatic glycosylation reaction, the electron transport chain in mitochondria, and membrane-bound NADPH oxidase (Brownlee, 2001; Harrison *et al*., 2003; Mohazzab *et al*., 1994). Chronic hyperglycemia is a cause of impairment of insulin biosynthesis and secretion. This process is called β – cell glucose toxicity which is often observed under diabetic conditions. In diabetic state, hyperglycemia and subsequent production of ROS decrease insulin gene expression and finally bring about apoptosis. In addition, ROS are induced and involved in the β – cell glucose toxicity. β – cells are rather vulnerable to ROS due to the relatively low expression of antioxidant enzymes such as catalase, glutathione peroxide and superoxide dismutase. Therefore it is likely that ROS are involved in β – cell deterioration found in diabetes (Evans *et al*., 2003). The potential mechanism of oxidative stress includes the reduction of antioxidant defense. In general, antioxidants such as phenolic compounds (tocopherols, flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids and ascorbic acid (Hall and Cuppett, 1997; Larson, 1988) compounds inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction. In this study, the levels of catalase, glutathione peroxidase and superoxide dismutase activities in plasma and liver tissues of diabetic group were significantly reduced and treatment with *B. sapida* stem bark aqueous extract generated the catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) activities not only on acute experiments but after 21 days of treatment. Decreased levels of CAT, GPx and SOD in the diabetic state may be due to inactivation caused by reactive oxygen species. In treated groups the increased CAT specific activity could be due to higher production of H_2O_2. It is possible that CAT activity which in turn would protect SOD inactivation by H_2O_2 causes an increase in SOD activity. Increase in SOD activity would protect GPx and CAT against inactivation by superoxide anion (Blum and Fridovich, 1985). An increase in the level of reduced glutathione could thus be due to it been spared as a result of the protection offered by superoxide dismutase to glutathione peroxidase.

It is known that lipotoxicity is also involved in the deterioration of β – cell function found in diabetes. The increase in free radicals in diabetic condition is suggested to be due to the increased lipid peroxidation and the damage to antioxidant defense system. Protein glycation and glucose autoxidation can generate free radicals that catalyze the lipid peroxidation (Altan*et al*., 2006). Any compound, natural or synthetic, with antioxidant activity might totally or partially alleviate this damage. In this study, direct effects of aqueous extract of *B. sapida* stem bark on malondialdehyde (MDA) levels in diabetes group were found to be higher than those in control group ($P<0.05$), indicating free radical generation via lipid peroxidation. Treatment of diabetes with the aqueous extract of *B. sapida* stem bark caused an eventual reduction in the MDA levels in plasma and liver after 21 days of treatment. Furthermore, direct effects of aqueous extract of *B. sapida*extract on protein carbonyl levels in diabetes group were found to be higher than those in control group ($P<0.05$), indicating increased free radical generation via production of various kinds of glycated proteins such as glycosylated hemoglobin, albumin and lens. Treatment of diabetes with the aqueous extract of *B. sapida* stem bark caused a reduction in the levels of protein carbonyl in plasma and liver after 21 days of administration.

V. Conclusion

One of the major findings of this study is that oral administration of aqueous extract of *B. sapida* stem bark caused anti-hyperglycemic activity in alloxan-induced diabetes in experimental albino rats. The results also revealed that *B. sapida* stem bark aqueous extract caused a significant increase in the activities of catalase, glutathione peroxidase and superoxide dismutase in the plasma and liver of diabetic rats after 21 days of treatment. It is also observed that aqueous extract of *B. sapida* stem bark extract possess the capability of inhibiting both lipid and protein peroxidation in diabetes.

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Global Journal of Medical Research: B
Pharma, Drug Discovery, Toxicology & Medicine
Volume 17 Issue 1 Version 1.0 Year 2017
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Phytochemical Screening and *in Vitro* Antioxidant Activity of Aqueous Extract of *Blighia Sapida* Stem Bark

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Abstract- *Blighia sapida* is a plant belonging to the family of sapindaceae. In this study we aimed to carry the phytochemical screening of aqueous extract of *Blighia sapida* stem bark and evaluate its *in vitro* antioxidant activity. It was found that the aqueous extract of *Blighia sapida* stem bark showed the presence of tannin, saponin, flavonoid, alkaloid, phenols and ascorbic acid. It also contains trace elements zinc and selenium. Furthermore it showed some scavenging activity but not as such could be compared with the various standards used except for nitric oxide scavenging activity. These are indications that the aqueous extract of *Blighia sapida* contains some physiologically significant secondary metabolites and also possesses appreciable *in vitro* antioxidant activity.

Keywords: *blighia sapida, phytochemicals, antioxidant, metabolites.*

GJMR-B Classification: *NLMC Code: QV 744*

Strictly as per the compliance and regulations of:

Phytochemical Screening and *in Vitro* Antioxidant Activity of Aqueous Extract of *Blighia Sapida* Stem Bark

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Abstract- ***Blighia sapida*** **is a plant belonging to the family of sapindaceae. In this study we aimed to carry the phytochemical screening of aqueous extract of *Blighia sapida* stem bark and evaluate its *in vitro* antioxidant activity. It was found that the aqueous extract of *Blighia sapida* stem bark showed the presence of tannin, saponin, flavonoid, alkaloid, phenols and ascorbic acid. It also contains trace elements zinc and selenium. Furthermore it showed some scavenging activity but not as such could be compared with the various standards used except for nitric oxide scavenging activity. These are indications that the aqueous extract of*Blighia sapida* contains some physiologically significant secondary metabolites and also possesses appreciable *in vitro* antioxidant activity.**

Keywords: blighia sapida, phytochemicals, antioxidant, metabolites.

I. Introduction

Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. In fact, phytochemicals (or antioxidants) such as phenolic compounds (tocopherols, flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids and ascorbic acid (Hall and Cuppett, 1997; Larson, 1988) compounds inhibit, or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reaction.

Blighia sapida is a plant belonging to the family of Sapindaceae. It is commonly known as ackee. In Nigeria, it is called Gwanja Kusa (Hausa), Isin (Yoruba) and Okpu (Igbo). It is an evergreen tree of about 33 to 40ft (10-12m) with a dense crown of spreading branches. It is rather hand usually with a short trunk to 6ft (1.8m) in circumference. Its bark is grey and nearly smooth. The leaves are compound with three to five pairs of oblong, ovate-oblong, or elliptical leaflets 1.5-3.0cm long. The seed of the fruit is not edible, whereas the fleshy aril is edible. The fruit is known to contain saponins, which are hemolytic (Aderinola *et al.*, 2007).

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Most of the earlier studies on *Blighia sapida* have been on the nutritional qualities of the root (Abolaji *et al*, 2007) and the leaves as a dry season feed resource for West African dwarf goats in the Northern savanna zone of Nigeria (Aderinola *et al*, 2007). The repellant potential of the fruit part components against stored-product insect pests (Khan and Gumbs, 2003) as well as neutropenia and thrombocytopenia effects of the aqueous and lipid extracts of the unripe fruit have been investigated in mice (Gardiner *et al*, 1996). More recently, the physicochemical properties of the oil from the fruit of the species and toxicological evaluation of the oil – based diet in Wister rats have been investigated (Oladiji *et al*, 2009).

Tree bark is an important component of African traditional medicine as herbal medicine is still the main source of health care for the majority of Africans and in particular, Nigerians.There has been increasing demand for the use of plant products with therapeutic activity. The high cost, availability, uncertainty of use during pregnancy and undesirable side effects of synthetic drugs or drugs from other animal sources are some of the factors leading to a strong preference for drugs of plants origin.

This study is thus aimed at investigating the phytochemicals and *in vitro* antioxidant activity of the readily available *Blighia sapida* stem bark.

II. Materials and Methods

Chemicals: All chemicals used were of analytical grade and items are products of BDH and Sigma Chemical Ltd., UK and Roche Diagnostic, Germany.

Sourcing for the Tree Bark of Blighia sapida: A sizeable quantity of the tree bark of *Blighia sapida* was obtained from the compound of the Federal Polytechnic, Ado Ekiti, Nigeria.

Identification of Plant: The fruits and leaves of *Blighia sapida* plant were obtained from the compound of the Federal polytechnic, Ado Ekiti, Ekiti State, Nigeria and were used for the purpose of authentication of the identity of the plant at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria. The voucher number of identification is UIH624.

Processing of sample and preparation of extract: The sample obtained was air-dried at room temperature for

fifty-six (56) days until a constant weight was obtained. The air-dried tree bark of *Blighia sapida* was pulverized. 100g of the pulverized sample was extracted with 800ml of distilled water for seventy-two (72) hours in an extractor. The aqueous extract was obtained by filtering with Whatman filter paper and subsequently freeze-dried in Armfield freeze-drier for ten (10) days.

Qualitative Phytochemical Analysis: Chemical tests were carried out on the aqueous extract using modified standard procedures to identify the constituents as described by Sofowora(1993), Trease and Evans(1989) and Harborne(1973).

Quantitative Determination of Phytochemicals

Determination of Tannin: The tannin content of the extract was determined by using the modified procedure of Makkar (1994).

Determination of Saponin: The method used was that described by Obadoni and Ochuko (2001).

Determination of Flavonoid: The method of Boham and Kocipal-Abyazam (1994) was used.

Determination of Alkaloid: The total alkaloid content of the extract was determined using the method described by Harborne (1973).

Determination of Total Phenols: The total phenolic content was determined using the method described by Singleton and Rossi(1965) using Folin-Ciocalteu's phenol reagent.

Determination of Zinc and Selenium: The level of zinc and selenium were determined by the method described by AOAC (2006).

In vitro Antioxidant Assay

DPPH free radical scavenging assay: The hydrogen or radical scavenging properties of the extract was determined using the stable radical DPPH (2, 2-Diphenyl-1-picrylhydrazyl hydrate) according to the method proposed and described by Blois (1958).

Hydroxyl radical scavenging assay: Deoxyribose assay was used to determine the hydroxyl radical scavenging activity in an aqueous medium (Halliwell *et al.*, 1981).

Hydrogen peroxide decomposition assay: This activity was determined according to a method described by Long *et al.*, (1999).

Nitric oxide (NO) scavenging assay: At physiological pH, nitric oxide generated from aqueous sodium nitroprusside (SNP) solution interacts with oxygen to produce nitrite ions, which may be quantified by the Griess Illosvoy reaction (Garratt, 1964).

Ferric reducing antioxidant assay (FRAP): The FRAP assay uses antioxidants as reductants in a redox-linked colorimetric method with absorbance measured with a spectrophotometer (Benzie and Strain, 1999). The principle of this method was based on the reduction of a colourless ferric-tripyridyltriazine complex to its blue ferrous colour formed owing to the action of electron donation in the presence of antioxidants.

Determination of total antioxidant capacity: The total antioxidant capacity was determined in accordance with the method described by Prieto *et al.*, (1999).

Statistical Analysis: Data were expressed as mean ± S.E.M. of five replicates.

III. Results

Phytochemical screening of extract: The result of phytochemical screening of aqueous extract of *Blighia sapida* stem bark shows the presence of tannin, saponin, flavonoid, alkaloid, phenols and ascorbic acid. It also shows the presence of the trace elements zinc and selenium (Table 1 and 2).

In vitro antioxidant activity: In vitro antioxidant studies revealed that aqueous extract of *Blighia sapida* stem bark showed some scavenging activity by total antioxidant capacity (TAC) with the IC_{50} value of 16.63± 0.51μg/ml, DPPH radial scavenging activity with the IC_{50} value of 23,938± 169.28 μg/ml, total phenol of 5.47± 0.93 μg/ml GAE, hydrogen peroxide radical decomposition activity with the IC_{50} value of 5,133.50± 191.70 μg/ml, hydroxyl radical scavenging activity with IC_{50} value of 6904.00± 751.90 μglml and nitric oxide scavenging activity with IC_{50} value of 447.57± 153.28 μg/ml. However, apart from the nitric oxide scavenging activity value that compares with that of the standard (i.e. ascorbic acid), all other values are a lot higher than those of the standards used (Table 3).

Table 1: Qualitative analysis of the phytochemicals of the aqueous extract of *Blighia sapida* stem bark

Specie	Extract Type	TNN	SPN	FLV	STR	TPN	AKD	PHN
Blighia sapida (Stem bark)	Aqueous	+	+	+	+	+	+	+

KEY: + = Presence of constituent
– = Absence of constituent
TNN = Tannin
SPN = Saponin
FLV = Flavonoid
STR = Steroid
TPN = Terpenoid
AKD = Alkaloid
PHN = Phenol

Table 2: Concentrations of some phytochemicals, zinc and selenium in aqueous extract of *Blighia sapida* stem bark.

Species	Extract Type	Tannin	Saponin	Flavonoid	Alkaloid	Phenols	Ascorbic Add(mg/100g)	Zinc (ppm)	Selenium (ppm)
Bllghla saplda	Aqueous	22.0±1.24	21.3±3.30	13.3±1.86	17.3±4.70	5.47±0.93	22.1±6.02	19.6±0.03	<1.0

Table 3: *In vitro* antioxidant activity of *Blighia sapida* stem bark aqueous extract

EXTRACT TYPE	Total Antioxidant Capacity (TAC)	Ferric reducing antioxidant (FRAP)	DPPH free radical Scavenging	Total Phenol (GAE)	Hydrogen Peroxide	Hydroxyl radical	Nitric oxide
			IC_{50} Values (μg/ml)				
Aqueous	16.63 ± 0.81	9.13 ± 0.51	23938.00 ± 160.28	5.47 ± 0.93	5133.50 ± 191.70	6904.00 ± 751.90	447.57 ± 153.28
			Standards				
Ascorbic acid	–	–	69.19 ± 5.02	–	123.41 ± 4.84	–	264.08 ± 11.07
Butylated Hydroxytoluene (BHT)	–	–	–	–	–	139.73 ± 31.31	–

Values are Mean±S.E.M of five determinations

IV. Discussion

Antioxidants such as phenolic compounds (tocopherols flavonoids, and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids and ascorbic acid (Hall and Cuppett, 1997; Larson, 1988) compounds inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions.

In this study, the metabolites shown in Table 1 were known to show biological activity as well as exhibiting physiological activity (Sofowora, 1993). Flavonoids are potent water-soluble antioxidants and free radial scavengers which prevent oxidative cell damage and have strong anticancer activity (Okwu*et al.*, 2006). Flavonoids also lower the risk of heart disease. Saponins are capable of neutralizing some enzymes in the intestine that can become harmful, building the immune system and promoting wound healing. Alkaloids have been documented to possess analgesic, antispasmodic and bactericidal effects. Tannins hasten the healing of wounds and inflamed mucuous membrane (Okwu *et al.*, 2006). The presence of these phytochemicals support the medicinal use of *Blighia sapida* (Saidu, 2012).

Zinc and selenium are the trace elements that have been found to be cofactors of antioxidant enzymes such as glutathione peroxidase and superoxide dismutase (Weydert and Cullen, 2009).

In vitro antioxidant studies revealed that aqueous extract of *Blighia sapida* stem bark only showed a comparative scavenging activity when compared to the standard (i.e. ascorbic acid), by nitric oxide scavenging activity. All other parameters considered in the *in vitro* antioxidant studies did not show promising results when compared with the standards.

V. Conclusion

A major finding of the study is that aqueous extract of *Blighia sapida* stem bark showed the presence of metabolites such as tannin, saponin, flavonoid, alkaloid, phenols and ascorbic acid. It was also found to contain trace elements zinc and selenium. The results also revealed that *Blighia sapida* stem bark aqueous extract showed some *in vitro* antioxidant activity. Ofall the parameters studied, nitric oxide scavenging activity was the only one that showed a somewhat comparable IC_{50} to that of the standard while all others did not compare well with the chosen standards.

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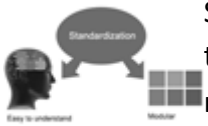

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Author Guidelines:

1. General,

2. Ethical Guidelines,

3. Submission of Manuscripts,

4. Manuscript's Category,

5. Structure and Format of Manuscript,

6. After Acceptance.

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- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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ISSN 9755896