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Nutritional Content, Phytochemical Evaluation and Antipyretic Effect of Methanol Leaf Extract of *Senna siamea* (Kassod tree)

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ABSTRACT

Senna siamea is a medicinal plant used locally for the treatment of typhoid fever, jaundice, abdominal pain, menstrual pain, and is also used to reduce sugar level in the blood.The study aimed at phytochemical screening; antipyretic activities of methanol leaf extracts of *Senna siamea*. Fresh leaf of *Senna siamea* was air-dried, pulverized and extracted using maceration method of extraction technique with methanol. The percentage proximate analysis was carried out on the dry matter has highest percentage (88.50), carbohydrate has (64.84%), followed by fibre content (10.00%), moisture content (11.50%), ash content (5.00%), protein content (8.66%) and fat or lipid content (1.0%) respectively. The phytochemical studies of the methanol leaf extract of *Senna siamea* revealed the presence of some chemical compounds such as alkaloids, flavonoids, cardiac glycosides, tannins, saponins, and terpenoids. The LD_{50} of the stem bark extract was ≥5000 mg/kg. The percentage changes in average rectal temperature (^oC) from induction of pyrexia to 2 hours after treatment with methanol leaf extract of *Senna siemea*. There was a dose dependent increase in percentage change in percentage change in rectal temperature, with the negative control showing the lowest (0.52%) change in rectal temperature. Extract dose of 100 mg/kg, 200 mg/kg and 400 mg/kg had a percentage decrease in rectal temperature of 0.85%, 2.07% and 3.26% respectively. However, the positive control had the highest percentage change in rectal temperature of 5.28 % when compared to the extract treatments. The leaf extracts decreased the rectal temperature of Brewer-induced pyrexia in albino rats, which was more effective at 200 mg/kg. Thus, this study has scientifically justified that the plant poses a degree of action on central nervous system thereby acting as in suppressing fever. This provides validity for the use of the plant locally for the management and treatment of fever related health problems.

Keywords: *Senna siamea*, proximate content, acute toxicity and antipyretic effect

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INTRODUCTION

Medicinal plants used in West Africa are as old as the duration of human settlement in the region [38, 29]. These can be either any part of the plant like bark, leaves, flowers, roots, fruits, seeds etc. [25]. The pharmacological properties of plants are immense: remedies made from plants play an important role in the health people leaving in the rural areas [22]. The availability, low cost and accessibility of these plants in Tropical and Sub-tropical Africa coupled with the global crisis of drug resistance incidences, adverse effects amongst other appalling negative scientific reports of conventional drugs make it convenient for in-depth survey of medicinal plants from this part of the world [31].

Chemicals referred to an active phytochemical include terpenes, flavonoid, bioflavonoid, benzophonones, xanthenes as well as some metabolites such as tannins, saponins, cyanates, oxalate and anthrax quinones [24,4].

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They protect plant from disease and damage and contribute to the plant's colour, aroma and flavour. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called phytochemicals [15].

METHODOLOGY

Sample Collection, Identification and Preparation

Fresh leaves of the plant, *Senna siamea* were collect from University of Maiduguri, Borno State, Nigeria. The leaf was identified and authenticated to be *Senna siamea* by a Plant Taxonomist in the Department of Biological Sciences, University of Maiduguri, Borno State, Nigeria. It was given a voucher specimen number 547A and deposited at the Postgraduate Research Laboratory of the Department of Chemistry, Faculty of Science, University of Maiduguri.

The leaves were cleaned by hand picking foreign materials and air-dried under shade at room temperature for seven days and pulverized using mortar and pestle and then subjected to the following analysis.

Sample preparation and proximate analysis

The air-dried samples were manually screened and crushed using a wooden mortar and pestle and stored in a dessicator. Two grammes (2 g) each of the crushed samples were processed for analysis of various parameters according to the Association of Official Analytical Chemists (AOAC, 1990; AOCS, 2000) methods. The proximate analysis such as moisture, ash, crude lipid content, crude fibre, nitrogen free extracts, crude protein and carbohydrates (by difference of the ethanol extract) of the dried samples were determined using AOAC methods [3]. The moisture and ash contents were determined using weight difference method. Crude fibre content was estimated from the loss of weight of the crucible and its content on ignition. Carbohydrate was determined by differential method when the sum of the percentages of moisture, ash, crude lipid / fat, crude protein and crude fibre content was subtracted from 100 [37].

The nitrogen value, which is the precursor for protein of a substance, was determined by micro-Kjehldahl method, involving digestions, distillation and titration of the sample [37]. The nitrogen value was converted to protein by multiplying nitrogen value with a factor of 6.25. The determination of crude lipid content of the samples was done using soxhlet extractor type of the direct solvent extraction method. The solvent used was n-hexane (boiling range 40-60^{o}C). The total carbohydrate (CHO) content was determined by difference, as the sum of the % Moisture, % Ash, % Crude Lipid / fat, % Crude Protein and % Crude Fibre which was subtracted from 100 [37]. All the proximate values were presented as percentages [3].

Plant Extraction

The powdered leaves of *Senna siamea* (500g) were extracted exhaustively by maceration using methanol. The crude extract was concentrated to dryness at reduced pressure in a vacuum using a rotary evaporator at 40^{o} C.

The extracts were weighed, labeled and subjected to phytochemicals screening. It was then exhaustively defatted using n-hexane.

Phytochemicals Screening

The screening was done in accordance with the standard protocol describe by [17]. The extract was screened for the presence of alkaloids, tannins, flavonoids, saponins, anthraquinones, terpenoids, cardiac glycosides and carbohydrate.

Experimental Animals

All the experiments performed on laboratory animals in this study followed the standard procedure for the treatment of animals. The animals were handled according to the International Guiding Principle for Biomedical Research involving animals, [13].

Acute Toxicity Evaluation (LD_{50})

The acute toxicity (LD_{50}) of the crude methanol stem bark extract was determined using standard conventional procedure as described by [48]. In this study, two different routes of administration were considered; the oral and intraperitoneal. In phase I, rats were divided into 3 groups of three rats each for each route (a total of nine rats) and then treated with the crude methanol extract at doses of 10, 100 and 1000 mg/kg bd. wt. intraperitoneally and orally and observed for 24 hours for mortality. In the phase II, the animals of each group (for each route) were divided into three groups of one animal each and the methanol extract was administered at doses that were determined after the phase I. The rats were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for 7 days. The LD_{50} was then calculated using the formula:

$LD_{50} = \sqrt{a \times b}$

Where

a = least dose that killed a rat

b = highest dose that did not kill a rat

Antipyretic Activity Study

Method described by [6] was used for this study. A total of twenty (20) rats were used for this study. The rats were divided into five (5) groups of four rats for the study on the stem bark methanol extract. Yeast suspension of 200mg/kg was administered intraperitoneally. The grouping was as follows:

Group 1: negative control (yeast only)

Group 2: positive control (yeast + Paracetamol 100mg/kg)

Group 3: yeast + extract 100 mg/kg

Group 4: yeast + extract 200 mg/kg

Group 5: yeast + extract 400 mg/kg

A total of twenty (20) rats were used for the study on the stem bark extract. Brewer's yeast (200 mg/kg) was administered intraperitoneally to the rats after measuring their baseline rectal temperatures. The rectal temperature for each rat was then re-measured and recorded after 8 hours. Only rats that showed an increase in temperature of at least 0.5^{o} C were used. Group 1 was not treated after the induction of pyrexia and served as negative control. Group 2 was treated with 100 mg/kg paracetamol administered orally using a naso-gastric tube and this served as the positive control. Group 3 was treated with 100 mg/kg of the extract administered orally (stem bark methanol extract). Group 4 was treated with 200 mg/kg of the extract administered orally (stem bark methanol extract). Group 5 was treated with 400 mg/kg of the extract administered orally (stem bark methanol extract). Rectal temperature measurements were taken 30 minutes, 1 hour and 2 hours following treatment.

A total of sixty-nine (69) albino rats (100-180 g) and twenty-five (25) mice (20-28 g) of both sexes were purchased from the Animal House of the Faculty of Pharmacy, University of Maiduguri, Borno State. They were housed in clean plastic, well-ventilated cages with saw dust as beddings under 12 hrs light/12 hours' dark cycle conditions of normal room temperature and humidity in the Pharmacology, Physiology and Biochemistry Laboratory, Faculty of Veterinary Medicine, University of Maiduguri for the analysis. They were fed with standard feed (ECWA, Jos) and allowed water *ad libitum.*

RESULTS

Proximate Analysis of *Senna siamea* Stem Bark

The percentage proximate analysis reveal that carbohydrate has the highest percentage (65.80 %), followed by fibre content (26.00%), moisture content (3.65%), ash content (2.00%), protein content (2.55%) and fat or lipid content (1.0 %) respectively.

Phytochemical components of dried leaves and methanol leaf extract of *Senna siamea*

The preliminary phytochemical screening of the dry matter and methanol leaf extracts of *Senna siamea,* the dry matter revealed the presence of phytochemicals such as flavonoids, terpenoids, steroidal nucleus, alkaloid, saponins and tannins. Methanol leaf extract has phytochemicals as tannins, triterpenes, saponnins, alkaloid and steroidal nucleus. The result of the phytochemical screening of the gradient extraction is shown in Table 1.

Table 1: The preliminary phytochemical constituents of the dry matter and methanol leaf extracts of *Senna siamea.*

S/N	Phytochemical Test	Dry Matter	Methanol Extract
1	**Test Tor Carbohydrates**		
I	General test-Molish	+	+
II	Test for monosaccharides-Barfoed	-	-
III	Test for reducing sugar-fehling test	-	-
IV	Test for combined reducing sugar	-	-
V	Test for ketoses	+	+
VI	Test for pentoses	_	_
2	**Test for Tannins**		
I	Ferric chloride test	+	+
II	Lead acetate	+	_
3	**Test for Phlobatannins**	_	_
4	**Test for Cardiac glycosides**		
I	Salkwoski test	+	+
II	Liebermann-burcharde test	+	+
5	**Test for Flavonoids**		
I	Shinoda's test	+	_
II	Ferric chloride test	+	+
III	Lead acetate test	+	_
IV	Sodium hydroxide	_	_
6	**Test for Terpenes**	+	+
8	**Test for Saponnins**		
I	Frothing test	+	+
9	**Test for soluble starch**	_	_
10	**Test for Alkaloids**		
I	Dragendroff's reagent	+	+
II	Meyer's reagent	+	+
11	**Test for steroidal nucleus**		
I	Keller- killiani's test	+	+

Key: + = detected; = Not detected

Acute Toxicity Evaluation (LD_{50})

The acute toxicity (LD_{50}) of the crude leaf extracts of methanol were determined using standard conventional procedure as described by [48]. In this study, two different routes of administration were considered; the oral and intraperitoneal. In phase I, rats were divided into 3 groups of three rats each for each route (a total of nine rats) and then treated with the crude methanol extract at doses of 10, 100 and 1000 mg/kg bd. wt. intraperitoneally and orally and observed for 24 hours for mortality. In the phase II, the animals of each group (for each route) were divided into three groups of one animal each and the methanol extract was administered at doses that were determined after the phase I. The rats were observed for signs of toxicity and mortality for the first critical four hours and thereafter daily for 7 days. The LD_{50} was then calculated using the formula:

$LD_{50} = \sqrt{a \times b}$

Where, a = least dose that killed a rat

b = highest dose that did not kill a rat

Table 2: Acute Toxicity of Methanol leaf Extract of *Senna siamea* in Rats

Phase	Dose (mg/kg)	No. of rat	Mortality rate	
			Oral route	IP route
I	10	3	0/3	0/3
	100	3	0/3	0/3
	1000	3	0/3	0/3
II	1600	1	0/1	0/1
	2900	1	0/1	0/1
	5000	1	0/1	0/1

$LD_{50} = \sqrt{ab}$

Where, a = least dose that killed a rat, b = highest doses that did not kill a rat

$= \sqrt{5000 \times 2900} = 3807$ mg/kg

Antipyretic Study of the Effect of Methanol Stem Bark Extract of *Senna siemea*

Effect of methanol Stem Bark Extract of *Senna siemea* on Yeast-induced Pyrexia

Table 3 shows the antipyretic effect of the methanol stem bark extract of *Senna siemea*. There was a dose dependent decrease in rectal temperature at 1 hr when doses of 100, 200 and 400 mg/kg were administered (38.45, 38.15 and 37.97^{o}C). No significant difference was observed at 30 min. and 1 hr after treatment (38.63, 38.40, 38.43^{o}C; 38.45, 38.15, 37.97^{o}C) respectively when compared to the rectal temperatures observed on induction of pyrexia. Result obtained after 2 hrs of treatment (38.33, 37.70, 37.95^{o}C) shows a significant difference ($p<0.05$) between the extract doses of 250 mg/kg (38.33^{o}C) when compared to the synthetic drug (paracetamol) 100 mg/kg (36.75^{o}C). However, there was no significant difference between the groups treated with higher doses of the extract (200 mg/kg and 400 mg/kg) when compared with the synthetic drug (paracetamol) 100 mg/kg.

Percentage Change in Average Rectal Temperature (^{o}C) from Induction of Pyrexia to 2 hours after Treatment with Methanol Stem Bark Extract of *Senna siemea*

Table 3 shows the percentage change in average rectal temperature (^{o}C) from induction of pyrexia to 2 hours after treatment with ethanol stem bark extract of *Senna siemea*. There was a dose dependent increase in percentage change in percentage change in rectal temperature, with the negative control showing the lowest (0.52 %) change in rectal temperature. Extract dose of 100 mg/kg, 200 mg/kg and 400 mg/kg had a percentage decrease in rectal temperature of 0.85%, 2.07% and 3.26% respectively. However, the positive control had the highest percentage change in rectal temperature of 5.28 % when compared to the extract treatments.

Table 3: Effect of Methanol Stem Extract of *Senna siemea* on Yeast-induced Pyrexia

Treatment groups	Dose (mg/kg)	Average Rectal Temperature (^{o}C)		Average Rectal Temp. after Administration of Drug (^{o}C)		
		Baseline	8 hrs after yeast administration	30 min.	60 min.	120 min.
Negative Control	-	36.40±0.32	38.63±0.40	38.45±0.29	38.80±0.20	38.83±0.36*
Senna siemea extract	100	36.00±0.33	38.66±0.18	38.63±0.11	38.45±0.22	38.33±0.29
Senna siemea extract	200	35.55±0.79	38.50±0.23	38.40±0.15	38.15±0.13	37.70±0.07*
Senna siemea extract	400	35.10±0.90	39.23±0.18	38.43±0.17	37.97±0.19	37.95±0.03
Positive Control – Paracetamol	100	37.20±0.44	38.80±0.04	38.25±0.13	38.20±0.25	36.75±0.80*

Results are in mean±standard error of mean (SEM); n=4; data with the same superscript are statistically significant ($p<0.05$)

DISCUSSION

The proximate analysis of the stem bark of *Senna siemea* showed that the moisture content was 3.65%. This suggests that the stem bark will have a long shelf life [36], the low moisture content could prevent microbial spoilage and pest attack during storage for medicinal purpose. Ash content of 5.00% in the stem bark shows that it could have an appreciable quantity of mineral elements [21]. The low crude fat (1.0%) of the stem bark could give the seed an extra advantage over some seeds and could show that it is a good source of fat which provides energy. The high amount of crude fiber in the stem bark, 10.00% means that it is a source of dietary fiber, which is essential for good bowel movement and could help in preventing obesity, diabetes, cancer of the colon and other ailments of the gastrointestinal tract of human [21]. The crude carbohydrate 64.840 % could be a good source of energy and thus a useful supplement in animal feed formulation and human diet [21].

The phytochemical studies of the methanol leaf extracts of *Senna siamea* revealed some useful chemical compounds such as flavonoids, cardiac glycosides, tannins, saponins, terpenoids and alkaloids. Flavonoids exhibit several biological effects such as antihepatotoxic, anti-inflammatory and antiulcer activity [8, 12]. Saponins have been reported to possess insecticidal activity [20], antitumorigenic effect [47], molluscicide effect [26], spermicidal [18], anxiolytic [10] (Chakraborty *et al*., 2010) and anti-bacterial activities [23]. Terpenes have been reported to possess important biological activities, such as analgesic [27,28], anticonvulsant [39], cardiovascular [40] antimalarial and antibacterial effects [17]. The saponin also exhibits antimicrobial [33], antioxidant [41] and anti-inflammatory activities [19]. The presence of saponins, steroids and triterpenoids in the plant extracts of *Senna siamea* supports the claim that these compounds have anti-inflammatory properties since saponins, steroids and triterpenoids have been found in other natural products with anti-inflammatory properties [32]. Alkaloids have pharmacological applications as anesthetics and CNS stimulants [35]. Other important alkaloids of plant origin include the addictive stimulants, caffeine, nicotine (III), bufotenin (IV), codeine, atropine, morphine, ergotamine, cocaine, nicotine and ephedrine [35]. The extract potently and significantly prolonged reaction time in mice subjected to thermal stimuli, indicative of an analgesic effect, comparable with the opioid agonist pentazocine. The tail immersion test of nociception screens for substances with central nervous system activity [42]. The hot plate test however, does not discriminate between central analgesics and muscle relaxants/sedatives, which also prolong reaction time in the hot plate test [43]. Anti-nociceptive model; tail immersion test was used to evaluate the analgesic activity, since tests of analgesic drugs commonly measure nociception and involve the reaction of animals to painful stimuli [44]. The stimulus may be thermal (tail immersion or hot plate tests), chemical (acetic acid-induced writhing or formalin tests) or mechanical (tail or paw pressure tests) [34]. The methanol leaf extract showed a dose-dependent and significant ($P < 0.001$) increase in the pain threshold post-treatment with dose of extracts in the tail immersion test. The effects of the extracts were significantly ($P < 0.001$) lower than those produced by pentazocine in the same tests. The tail immersion has been used to study centrally acting analgesics [45, 7]. In these tests, the nociceptors are sensitised by sensory nerves and the involvement of endogenous substances such as prostaglandins are minimized. Thus from the results, it may be concluded that the analgesic activity of *Senna siamea* may be fully mediated through central mechanism. The abdominal constriction method used in evaluation of the effect of the plant extract is a very sensitive one and can detect antinociceptive effect of a substance at a dose that cannot be detected by other methods such as tail-flick test [46, 14]. Inhibition of acetic acid-induced writhing in mice by extract (200 and 400 mg/kg) suggested that the analgesic effect of the extract may be peripherally mediated via the inhibition of the synthesis and release of prostaglandins [46].The acetic acid induced mice writhing test has been used extensively to qualify analgesic agents that have peripheral analgesic activity [30]. Writhing induced by chemical substances injected intraperitoneally, is due to sensitization of nociceptors by prostaglandins.

REFERENCES

[1]. AOAC. (Association of Official Analytical Chemists) (2005). *Official methods of analysis.* 15th Association of Official Analysis Chemist Washinton D.C Pp 774-784

[2]. AOAC. (2000). Official Methods of Analysis, 15th ed. Washington, DC: Association of Official Analytical Chemists. *J. Nut. Biochem,* 6(7), 131.

[3]. AOAC., (Association of Official Analytical Chemists) (2016). *Official methods of analysis*, 20th Ed. Washington DC, USA., 120.

[4]. Abdulrahman FI, Akan JC, Sodipo OA, Onyeyili PA. (2010). Effect of aqueous root-bark extract of *Vitex doniana* sweet on hematological parameters in rats, J. Am. Sci, 6, 8-12.

[5]. Asaolu MF., 2003. Chemical composition and phytochemical screening of the seeds of Garcinia kola. Pakistan J. Sci. Ind. Res., 46, 145-147.

[6]. Backhouse N, Delporte C, Negret R, Salinas P, Pinto A, Aravena S, Cassels BK., (1996). Antiinflammatory and antipyretic activities of *Cuscuta chilensis, Cestrum parqui* and *Psorolea glandosa. Intl J. Pharmacog*. 34, 53-57

[7]. Bachlav RS, Gulecha VS, Upasani CD. (2009). *Indian Journal Pharmacology*. 41(4), pp158–161.

[8]. Bors W, Heller W, Michel C, Saran M., 1990. Flavonoids as antioxidants: Determination of radical scavenging efficiencies. *Methods in Enzymology*, 186, pp343-355.

[9]. Cardoso D, Pennington RT, De Queiroz LP, Boatwright JS, Van Wykd BE, Wojciechowskie MF, Lavin M. (2013). Reconstructing the deep-branching relationships of the papilionoid legumes, *South African Journal of Botany,* 89, 58–75. (Cardoso, *et al*., 2013)

[10]. Chakraborty A, Amudha P, Geetha M, Surjit SN. (2010). Evaluation of anxiolytic activity of methanolic extract of *Sapindus mukorossi* Gaertn. in mice. *International Journal of Pharmacy and Biological Science*, 1, 1-8.

[11]. Chattopadhyay D, Arunachalam G, Ghosh L, Mandal A, Bhattacharya SK. (2005)). Antipyretic activity of Alstonia macrophylla Wall ex A. DC: An ethnomedicine of Andaman Islands. *Jounal of Pharmacy and Pharmaceutical Sciences,* 8, 558-564.

[12]. Colerige SPO, Thomas P, Scurr JH, Dormandy JA. (1980). Causes of various ulceration, a new hypothesis, *Britain Medical Journal*, pp1726-1727.

[13]. CIOMS, ICLAS. (2012), Principles find medical sciences and the International Council for Laboratory Animal Science. Guiding Principles for Biomedical Research Involving Animals. http: / / idas. Org / wp-content / uploads / 2013 / 03 / C IOMS-ICLAS. Access Date: 22 / 4 / 2015.

[14]. Collier HOJ, Dinneen LG, Johnson CA, Schneider C. (1968). The abdominal constriction response by analgesic drugs in mouse. *British Journal of Pharmacology*, 32, pp295-301.

[15]. Deepak K, Rupali S, Mahesh K., 2016. An Overview of Major Classes of Phytochenicals: Their Types and Role in Disease Prevention. *Hislopia journal*, 9(1 / 2), 1-11.

[16]. De Sousa DP, Quintans-Jr LJ, Almeida RN. (2007). Evaluation of the anticonvulsant activity of alpha-Terpineol. *Pharmaceutical Biology*, 45, pp.69–70.

[17]. Evans WC. (2009). *Trease and Evans Pharmacognosy.* 16th Edition. Saunders Publishers, London. pp. 42–44, 221–229.

[18]. Garg S, Taluja V, Upadhyay M, Talwar GP. (1993). Studies on contraceptive efficacy of Praneem polyherbal cream. *Contraception*, 48, pp591-596.

[19]. Gepdireman A, Mshvildadze V, Suleyman H, Elias R. (2005). Acute anti-inflammatory activity of four saponins isolated from ivy: alphahederin, hederasaponin-C, hederacolchiside-E and hederacolchiside-F in carrageenan-induced rat paw edema. phytomedicine, 12(6-7), 440-444

[20]. Geyter ED, Geelen D, Smagghe G. (2007). First results on the insecticidal action of saponins. *Community of Agriculture and Applied Biological Science*, 72, 645-648.

[21]. Gadanya AM, Atiku MK, Otaigbe BO., 2014. Proximate and elemental

analysis of Baobab (*Adansonia digitata*) seed. *Int. J. Anal. Biochem. Res*, 1(1), 1 – 4.

[22]. Grabley R, Thirick R, Robak J. (1999). On the mechanism of antithrombotic action of flavonoids. *Biochemical Pharmacology*, 36, 317-321.

[23]. Ibrahim M, Khan A, Tiwari SK, Habeeb MA, Khaja MN, Habibullah CM. (2006). Anti-microbial activity of *Sapindus mukorossi* and *Rheum modi* extracts against Helicobacter pylori: in vitro and in vivo studies. *World Journal of Gastroenterology*, 12, 7136-42.

[24]. Iwu MM. (1993). *Handbook of African Medicinal Plants*. 1st edition. CRC Press Inc. Boca.

[25]. Newman DJ, Cragg GM., 2011. Natural products as sources of new drugs over the last 25 years, J. Nat. Prod. 70, 461–477.

[26]. Huang T, Hantash AEJ, Lewi G. (2010). Evaluation of Narcotic (Opioid Like) Analgesic Activities of Medicinal Plants. *European Journal of Scientific Research*, 33(1), 179-182.

[27]. Guimaraes AG, Quintans JSS, Quintans-Jr LJ., 2013. Monoterpenes with analgesic activity-A systematic review. *Phytotherapy Research*, 27, 2013, 1–15.

[28]. Quintans JSS, Menezes PP, Santos MRV, Bonjardim LR.

[29]. Almeida JRGS, Gelain DP. (2013). Improvement of p-cymene antinociceptive and anti-inflammatory effects by inclusion inb-cyclodextrin. *Phytomedicine*. 20, 436–440.

[30]. Sodipo OA, Abdulrahman FI, Akan JC, Akinniyi JA. (2008). Phytochemical Screening and Elemental Constituents of the Fruit of *Solanum macrocopum* Linn. *Continental Journal of Applied Science*, 3, 88-97.

[31]. Neves SLA, Freitas AL, Sousa BW, Rocha ML, Correia MV, Sampaio DA, Viana GS. (2007). Antinociceptive properties in mice of lecithin isolated from the marine alga *Amansia multifida Lamouroux*. *Bra. J. Med. Biol. Res.* 40, 127-134.

[32]. Usman H, Abdulrahman FI, Kaita HA, Khan IZ. (2013). Antibacterial effects of cyanogenic glucoside isolated from the stem bark of *Bauhinia rufescens* Lam Int. J. Biol. Chem. Sci. 7(5), 2139-2150.

[33]. Yeonju L, Jae-Chul J, Zulfiqar A, Ikhlas AK Seikwan OH. (2012). Anti-inlammatory effect of triterpenes saponins isolated from Blue cohosh (*Caulophyllum thalictroides*). Hindawi Publishing Corporation; article ID798192, 2012, 8pp.

[34]. Chattopadhyay D, Arunachalam G, Ghosh L, Mandal A, Bhattacharya SK. (2005. Antipyretic activity of Alstonia macrophylla Wall ex A. DC: An ethnomedicine of Andaman Islands. *Jounal of Pharmacy and Pharmaceutical Sciences,* 8, 558-564.

[35]. George KA, Eric W, David DO, George AK. (2009). Antinociceptive effects of Newbouldia laevis (P. Beauv.) stem bark extract in a rat moedel. *Pharmacognosy Magazine*. 17, pp49–54.

[36]. Madziga HA, Sanni S, Sandabe UK. (2010. Phytochemical and elemental analysis of *Acalypha wilkesiana* leaf. *Journal of American Science*, 6(11), 510-514.

[37]. Oyenuga VA. (1968). *Nigerian Food and Feedings Stuffs. Theire Chemistry and Nutritive value*. Nigerian University Press. Ibadan, pp.99.

[38]. Pearson G. (1976). Determination of Nitrogen Value, Using Micro-Kjiehldahl Method, *Chemistry of Natural Products. 16*, 165 – 169.

[39]. Abdulrahman FI. (2004). Studies on the Chemical Contents and Pharmacological Activities of the Root-Bark Extract of *Vitex doniana* (Black Plum). *Ph.D. Thesis,* (Unpublished). *University of Maiduguri,* Maiduguri, Nigeria.

[40]. De Sousa DP, Quintans-Jr LJ, Almeida RN. (2007). Evaluation of the anticonvulsant activity of alfa-Terpineol. *Pharmaceut. Biol*, 45, 69–70.

[41]. Silva-Filho JC, Oliveira NNPM, Arcanjo, DDR, Quintans-Jr LJ, Cavalcanti SCH, Santos M. R. (2012). Investigation of mechanisms involved in (-)-borneol-induced vasorelaxant response on rat thoracic aorta. *Basic Clin. Pharmacol. Toxicol.*, 110, 171–177.

[42]. Gryglewski RJ, Korbut R, and Robak J. (1987). On the mechanism of antithrombotic action of flavonoids.

Biochemical Pharmacology, 36, 317-321.

[43]. Sulaiman, M.R., Tengku-Mohamad, T.A.S., Shaik M.W.M., Moin, S., Yusof, M., Mokhtar AF, Zakaria ZA, Israf DA, Lajis N. (2010). Antinociceptive Activity of the Essential Oil of *Zingiberzerumbet*. *Planta Medica,* 76 (2), 107–112.

[44]. Vogel H.G. (2005). Drug Discovery and Evaluation: Pharmacological Assays. Third Edition. Springer. Aalen- Germany. 1164-1165.

[45]. Rang HP, Dale MM, Ritter JM, Moore PK. (2003)). Pharmacology. 5th edn. New Delhi India, Elsevier Science Ltd;

[46]. Wolfe MM, Lichtenstein DR, Singh G. (1999). Gastrointestinal toxicity of NSAIDs. *TheNew England Journal Medicine*; 340, 1888-1899.

[47]. Koster R, Anderson M, De-Beer EJ. (1959). Acetic acid for analgesic screening. *Federation Proceedings*, 18, pp412-418.

[48]. Mamta S, Jyoti S, Rajeev N, Dharmendra S, Abhishek G. (2013). Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry*, 1(6), 168-182.

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