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Phytochemical, Antioxidant and Analgesic activity of Stem Bark Methanol extract of *Sclerocarya birrea* (Anacardiaceae)

[1*]Namadina, M. M., Haruna, H[1]., Ibrahim, S[1]., Sadau, S.B[2]., Sunusi, U[3]., Kamal, R.M[4]., Nuhu, Y[1]., Balarabe, S[5]., Sani, S.B[6] ., Muttaka, A[7]., Nuhu, A.A[9]., Aminu, I.M[9]., Aminu, A[8]., Yakubu, N[1]., Yakasai, B.D.[10] and Abubakar, K.D.[11]

[1]Department of Plant Biology, Bayero University, Kano
[2] Department of Science Technology Education, Bayero University, Kano
[3]Department of Biochemistry, Faculty of Basic Medical Science, Bayero University, Kano
[4]Department of Pharmacology, Federal University, Dutse, Jigawa State, Nigeria
[5]Department of Integrated Science, Federal College of Education, Kano
[6] Department of Biology, Kano University of Science and Technology, Wudil
[7]Department of Biochemistry, Federal University Gusau
[8]Department of Trypanosomiasis Research, Kano State Liaison Office Infectious Diseases Hospital
[9]Department of Biological Sciences, Yusuf Maitama Sule University, Kano State
[10] Department of Biological Sciences, Bayero University, Kano
[11]Department of Biological Sciences, Federal University Dutsinma
Corresponding author: hajiyaiyalle@gmail.com, +2347035939668

Abstract
The study was aimed at establishing a safety profile, evaluating phytochemical constituents, antioxidant and analgesic properties of methanol stem bark extract of *Sclerocarya birrea*. Phytochemical, acute toxicity, antioxidant and analgesic studies was carried out using standard methods. The analgesic effect was investigated using acetic acid induced writhing test in mice. The extract was administered at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg. The extract was found to contain alkaloids, flavonoids, tannins, saponins, cardiac glycosides, and triterpenes. The extract was found to possess constituents that may be associated with its analgesic and antioxidant effects observed at doses tested. It was determined that methanol extract of *Sclerocarya birrea* stem bark possessed higher radical scavenging ability of 98.5% at the highest concentration of 1000 µg/mL and was compared with standard where it showed 99.7% activity. The DPPH radical scavenging ability of the extract showed the following trend Ascorbic acid < Methanol extract. The effects observed at 50 mg/kg and 100 mg/kg were more than that of 25 mg/kg of the extract. The effect observed in 100 mg/kg and 50mg/kg groups was comparable to that of the standard. The median lethal dose in rats was found to be above 5000 mg/kg. Collectively, this study provides scientific data for the use of *Sclerocarya birrea* in the treatment of pains, oxidative stress and contribute to the analgesic and antioxidant knowledge of this species. The bioactive constituents responsible for the pharmacological activities should be isolated and characterized

Keywords: Analgesic, Antioxidant properties, Pharmacological, Phytochemical constituents and *Sclerocarya birrea*
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Introduction

Plants used in traditional medicine are relatively safe, but some may have undesirable adverse effects which may be due to over dosage or certain factors and these may lead to toxicity and death (Okigbo *et al.,* 2009). The use of analgesics also known as pain killers is the most common method in treatment of pain. These pain medications may cause serious side effects in some individuals. Orthodox drugs used in the treatment of pain include, non-steroidal anti-inflammatory drugs, opioid and non-opioid analgesics (Feinberg *et al.,* 2012). Non-steroidal anti-inflammatory drugs such as aspirin, ibuprofen, diclofenac and naproxen in the treatment of mild to moderate pain, inflammation and pyrexia are limited by their significant side effects such as gastrointestinal tract irritation, blood disorders, liver damage, renal damage, tinnitus, hypersensitivity reactions etc (Mishra *et al* ., 2011).

Opioid analgesics, though very effective in chronic pain management, are associated with problems such as addiction and tolerance, and side effects such as constipation, weight gain and loss of libido. Opioids are also associated with dependence, and abrupt withdrawal can lead to withdrawal symptoms such as tiredness, diarrhoea, abdominal cramps and sweating (Stannard *et al.,* 2007). Majority of the world population depend on traditional medicine such as herbs for treatment of various ailments. Present day medicine was derived from herbal traditions (Ezeonwumelu *et al.,* 2012). The use of traditional medicine is rapidly growing; most people are working in the field of ethnomedicine due to accessibility and affordability. Hence, there is need for the establishment of toxicological profiles of these medicines (Salawu *et al.,* 2009).

The analgesic and antioxidant activities of *Sclerocarya birrea* have not been established scientifically. Problems associated with drugs used in fever, pain and inflammation are alarming, which necessitate the need for development of new drugs and variety of treatment option from bioactive constituents obtained from plants used in traditional medicine (Stark *et al.,* 2013).

Sclerocarya birrea {(A. Rich.)Hochst.}sub sp. *caffra* (Sond.) Kokwaro, Family: Anacardiaceae, commonly known as marula tree in English is a common and important tree in Africa with multifaceted uses recognized as a commercially, medicinally and culturally important plant species in the continent (Ojewole *et al.,* 2010). The common names in different languages are: Marula (English), Danya (Hausa). In Africa marula tree is widely distributed in savannah regions expanding from Gambia to Nigeria in the west, Cameroon in central Africa and to Sudan and Ethiopia from east (Berhaut, 1971). The aqueous and methanolic extracts of marula plant exhibited good anti-oxidant activities (Russo *et al.,* 2013). Dichloromethane and methanol extracts of *S. birrea* cortex exhibited strong anti-oxidant activity (Mayo *et al.,* 2010). Methanolic root extract possess anti-oxidant activity (Armentano *et al.,* 2015). Ojewole, (2004) reported that the aqueous extract of the stem bark of *S. birrea* exhibited low analgesic activity on mice induced with electrical heat pain compared with the standard drug (diclofenac).

Materials and Methods

Collection and Identification of Plant Materials

The stem bark of *Sclerocarya birrea* were collected from local farm in March, 2019 at Babura Local Government Area, Jigawa state. The plant was identified and authenticated in the Herbarium of the Plant Biology Department of Bayero University, Kano and was compared with a voucher specimen number BUKHAN435.

Preparation of Plant extracts

Stem bark powder (100 g) of *Sclerocarya birrea* was extracted successively in 1000 ml of methanol for 72hrs using cold maceration and the concentrate was evaporated to dryness on a water bath and stored in desiccator for further use.

Qualitative Phytochemical screening of the Methanolic extract of *Sclerocarya birrea* Stem bark

The methanol extract was subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described below.

Tests for carbohydrates

Molish's (General) Test for Carbohydrates: To 1 ml of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

Tests for Saponins

Frothing test: About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds. The tube was allowed to stand in a vertical position and was observed for 30 mins. A honeycomb froth that persists for 10-15mins indicates presence of saponin.

Test for Flavonoids

Shinoda Test: A portion of the extract was dissolved in 1-2ml of 50% methanol in the heat metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red color indicates presence of flavonoids (Evan, 2009).

Test for Alkaloid

Wagner's Test: Few drops of Wagner's reagents were added to a portion of the extract, whitish precipitate indicates the presence of alkaloid (Evans, 2009).

Test for Steroid and Triterpenes

Liebermann-Burchard's test: To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the presence of Triterpenes while blue or blue green indicates steroids (Evans, 2009).

Test for Cardiac Glycoside

Kella-killiani's test: A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observed carefully at the interphase for purple-brown ring, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 2009).

Test for Tannins

Ferric chloride test: To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate (Evans, 2009).

Test for Anthraquinones

Bontrager's test: To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5mins. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 2009).

Antioxidant Activity Procedure

The antioxidant activity of the plant extracts was measured in terms of radical scavenging ability, using a stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the modified method adopted from (Sani and Dailami, 2015). 200µl of 100µM methanol solution of DPPH were added to 100µL of various concentrations of the sample fractions in methanol (1000, 500, 250, 125, 62.5, 31.25, and 15.63µg/ml) and made to react in dark for 30mins time at room temperature. Absorbance of the blank, test and control were recorded at 517 nm. The experiment was performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of inhibition.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

The concentration corresponding to the 50% inhibition (IC_{50}) was determined using probit analysis by means of SPSS 16.0 software. The IC_{50} values obtained are compared with that of ascorbic acid as a standard antioxidant.

Analgesic studies

Acetic acid induced writhing in mice

Acetic acid induced writhing method described by (Koster *et al.*, 1959) was adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (Mishra *et al.*, 2011). 30 Swiss albino mice of both sexes were divided into five groups, 1 and 5 served as negative control (distilled water 10 ml/kg) and positive control (Piroxicam 20 mg/kg), while groups 2, 3, and 4 received 100 mg/kg, 50 mg/kg, and 25 mg/kg of the extract. Sixty minutes after treatment, the mice received 0.6% acetic acid (10ml/kg) interperitoneally to induce pain. 5minutes after acetic acid injection, the animals were observed and number of writhes by each mouse was counted for 15minutes. Percentage inhibition was calculated using the following formular:

$$\% \text{ inhibition} = \frac{\text{Average number of writhes (control)} - \text{Average number of writhes (test)}}{\text{Average number of writhes (control)}}$$

Acute Toxicity Studies (LD50**)**

The LD_{50} of the extract was determined using Lorke's method (1983). The study was carried out in two phases and animals (mice) were deprived of food for 16-18h prior to administration of the extract. In phase 1, three groups of three animals per group were used. The extract was administered orally in geometrically increasing doses (10mg/kg, 100mg/kg and 1000mg/kg). The treated animals were observed for four hours post administration for signs of toxicity. After 24 hours, phase 2 was initiated. In phase 2, four groups of one animal each were given the extract orally in geometrically increasing doses (1500 mg/kg, 2250 mg/kg, 3250 mg/kg and 5000 mg/kg). The animals were then observed for signs of toxicity for the first 4 hours and mortality for 24hours. The arithmetic mean of the lowest dose that killed an animal and the highest dose that did not kill was taken as the mean lethal dose (LD_{50}) of the extract.

Results

Phytochemical screening of the methanol extract revealed the presence of alkaloid, flavonoids, saponins, glycoside, triterpenes, steroid, tannins and phenols while anthraquinones was absent.

The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend Ascorbic acid < Methanol extract.

Free radical scavenging ability of two extracts of *Sclerocarya birrea* stem bark was evaluated using DPPH radical. Ascorbic acid was used as positive control. It was determined that methanol extract of *Sclerocarya birrea* stem bark possessed higher radical scavenging ability of 98.5% at the highest concentration of 1000 µg/mL and was compared with standard where it showed 99.7% activity (Figure 1).

Table 1. Qualitative Phytochemical screening of methanolic stem bark extract of *Sclerocarya birrea*

Metabolite	Inference
Alkaloid	+
Flavonoid	+
Saponins	+
Cardiac glycoside	+
Tannins	+
Steroid	+
Triterpenes	+
Phenol	+
Anthraquinones	-
Carbohydrate	+

Table 2. TLC results of Methanolic extract of *Sclerocarya birrea* stem bark

Extract	Solvent system	Number of Spots	Distance of spots	RF-Value
S. longipedunculata (Methanol)	HE: EA (8:2)	3	5.0	0.20, 0.52, 0.58

Key: HE(Hexane), EA(Ethyl acetate). (Remove distance of spots? RF-Value is enough)

Table 3: Antioxidant Activities of the methanol extract of *Sclerocarya birrea* stem bark

Sample	IC_{50} (µg/mL)
Methanol extract	0.019
Ascorbic acid	0.79

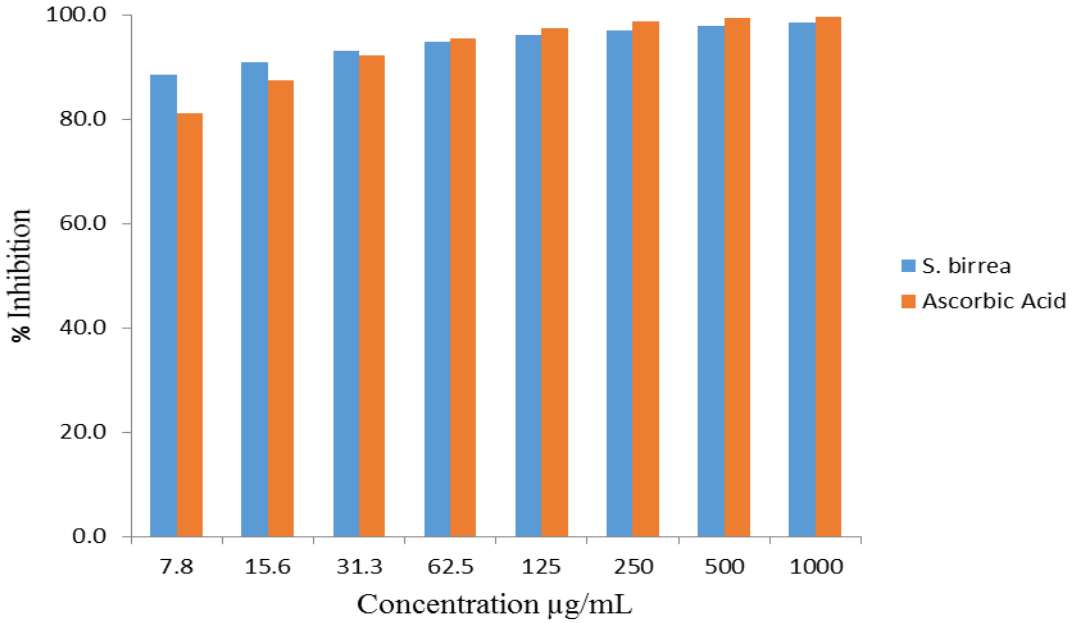

Figure 1. DPPH Antioxidant Activities of *Sclerocarya birrea* stem bark

Injection of acetic acid i.p. produced writhing, exhibited as an exaggerated distension of the abdomen combined with the outstretching of the hind limbs seen more in control mice pre-treated with normal saline.

The extract significantly decreased the number of writhes caused by acetic acid in a dose independent manner as shown in Table 4. The effects observed at 50 mg/kg and 100 mg/kg were more than that of 25 mg/kg of the extract. The effect observed in 100 mg/kg and 50mg/kg groups was comparable to that of the standard.

No death was recorded in the first phase of the study in rats. In the second phase, doses of 1500, 2250, 3250 and 5000mg/kg were used and no death was also recorded. The oral median lethal dose (LD50) for the methanol stem-extract of *Sclerocarya birrea* was therefore estimated to be greater than 5000mg/kg and no sign of behavioural changes were also observed.

Table 4: Effect of methanol stem bark extract of *Sclerocarya birrea* on Acetic Acid Induced writhing in mice

Treatment	Dose (mg/kg)	Mean No. of Writhes ± SEM	Inhibition (%)
Distilled water	10ml/kg	20.33±0.49	-
Piroxicam	10	3.83±0.31	81.2
Extract	25	6.17±0.54	69.7
Extract	50	2.67±0.33	86.9
Extract	100	1.83±0.31	90.1
LSD	-	0.79	-

Table 5. Acute toxicity studies of aqueous and methanolic stem bark extract of *Sclerocarya birrea*

Plant species	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24hrs
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

Discussion

These primary and secondary metabolites in plants have numerous functions. Crude, pure and isolated alkaloids and their synthetic derivatives have been used as analgesic, antispasmodic and bactericidal agents (Stary, 1998; Okwu and Okwu, 2004). Flavonoids have been shown to provide antibacterial, anti- inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity (Alan and Miller, 1996). Flavonoids are most commonly known for their antioxidant activity; they are the most common group of polyphenolic compounds in the human diet and are found ubiquitously in plants (Spencer *et al.,* 2008). Flavonoid also has immense antioxidant and anti-inflammatory activity because of its ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Okwu, 2004; Okwu and Josiah, 2006). Tanins have been used in the treatment of wounds especially those emanating from varicose ulcers and hemorrhoids (Njoku and Akumufula, 2007) and is able to stop bleeding during circumcision (*Edeoga et al.,* 2005). The phytochemical constituents especially the secondary metabolites could be useful as guide to chemotaxonomic markers (Jonathan and Tom, 2008) that will aid in chemo taxonomical classification system and further phylogenetic studies in Anacardiaceae family.

The thin layer chromatographic analysis of the methanol extracts was carried out on TLC plate precoated with analytical silica gel and developed in suitable solvents systems at different ratios, and it gave distinct and good degree of separations of phytochemicals. The methanol extract when developed in Hexane: Ethyl acetate (8:2) and sprayed with *p*-Anisaldehyde/H_2SO_4 reagent for visualization, it gave three spots of mostly purple colour alongside their Rf values. The Thin Layer Chromatography chemical screening is usually done to target isolation of new or very important constituent present in the plant extracts which has marked pharmacological activities and also serves as an important tool in recognizing how metabolite for isolation behaved and can be purified; hence, channeling scientific efforts towards the desired compound(s) and prevent waste of resources and time (Patra *et al.,*

2012). The success of separation of bio-molecules by chromatographic technique is markedly influence by the suitability of the separating solvent systems largely influences the successful and also rely upon an ideal range of partition coefficient (k) for each target compound(s) (Ito, 2005).

The trend of the inhibition of DPPH radical by the extract was concentration dependent with respect to the IC_{50} values (concentration of the extract to cause 50% inhibition), the DPPH radical scavenging ability of the extract showed the following trend Ascorbic acid < Methanol extract . It is interesting to note that the lower the IC_{50} value, the higher the scavenging activity of the plant extract (Sowunmi and Afolayan, 2015). The effect of antioxidants on DPPH radical scavenging is thought to be due to hydrogen donating ability. DPPH is a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Kannan *et al*., 2010). When these molecules are formed, the absorbance decreases and the DPPH solution decolourises from violet colour to pale yellow. The degree of discolouration is an indication that the plant extract has the potential to scavenge free radicals as a result of its ability of hydrogen donation. More yellowish colour of DPPH is an indicator stronger antioxidant activity of the extracts (Sowunmi and Afolayan, 2015).

The production of free radicals has been associated with various physiological and pathological events such as inflammation, aging, mutagenicity and carcinogenicity (Ozsoy *et al.,* 2008). Antioxidants are vital substances with the ability to protect the body from the damage caused by free radical-induced oxidative stress (Ozsoy *et al.,* 2008). Natural antioxidants present in medicinal and dietary plants have been implicated in the prevention of oxidative damage (Silva *et al.,* 2005). Flavonoids are phenolic compounds with potent metal chelating and free radical scavenging activities (Middleton *et al.*, 2000). Along with other natural antioxidant, vitamins and enzymes, they provide protection against free radicals by acting as antioxidants involved in scavenging reactive oxygen species (Varalakshmi *et al.*, 2011).

Acetic acid-induced writhing is among the sensitive methods for evaluating potential analgesic drugs or compounds that act peripherally. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, or turning of trunk (Mishra *et al.,* 2011). This model of response is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway. The acetic acid induced writhing model has been associated with increased level of prostaglandins (PGE2 and PGF2α) in peritoneal fluids as well as lipoxygenase products; this enhances inflammatory pain by increasing capillary permeability (Lakshman *et al.,* 2006; Khan *et al.,* 2010). The mechanism, by which any substance that inhibits these writhings causes analgesia, will be preferably by inhibition of prostaglandin synthesis, which is a peripheral mechanism of pain inhibition (Somachit and Shahid, 2003). The data presented suggests that the methanol extract of *Sclerocarya birrea* stem bark possess peripheral analgesic property. The extract at all doses was shown to have analgesic property as evidenced in the model used. The reduction in the number of writhes caused by the extract, suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of prostaglandins and other endogenous substances.

Acute toxicity studies of *S. birrea* stem bark was performed using Lorke (1983) method. With careful observations of experimental animals from the first 30 minutes up to the 14th day, it was revealed that there were no deaths and any sign of toxicity such as loss or increase in weight, tiredness, abdominal constrict convulsion, hyperactive, weakness, diarrhea or increased diuresis within the short and long term effect in rats dosed with 5000 mg/kg body weight of the *S. birrea* extracts (methanol). The outcome of the study of Alhassan *et al.,* (2014) gave an LD_{50} of 2000 mg/kg and this guided our choice of dose used (5000 mg/kg). The LD_{50} was found to be greater than 5000 mg/kg body weight orally, and this suggested that the extract has low

acute toxicity when administered orally. This may be attributed to the incomplete absorption brought about by inherent factors limiting absorption in the gastro intestinal tract (Dennis, 1984). The present study agrees with the work done by Ugbogu *et al*., (2016) and Adesegun *et al.,* (2016). Bruce, (2006) reported that any substance with LD_{50} estimated to be greater than 2000-5000 mg/kg body weight given orally could be considered to be of low toxicity and safe. Similarly, the chemical labelling and classification of acute systemic toxicity based on oral LD_{50} values recommended by the organisation of Economic Cooperation and Development (OECD, Paris, France) and (Walum, 1998) are as follows: less than 5 mg/kg: very toxic, greater than 5 but less than 50 mg/kg: toxic, greater than 50 but less than 500 mg/kg: harmful, and, greater than 500 but less than 2000 mg/kg: no label. The very high LD_{50} observed is not a conclusive finding about the safety of the extracts of *Sclerocarya birrea*, higher doses could be tested for better understanding of its effects if use for a long period of time and for proper recommendation on its future utilization (Ogbonnia *et al.*, 2011).

Conclusion

The methanol stem bark extract of *Sclerocarya* was found to possessed several bioactive constituents including flavonoids, saponins, tannins, cardiac glycosides among others, associated with potent pharmacological activities. The extract was found to possessed considerable analgesic and antioxidant properties at doses tested. This partly justifies the claim for the traditional use of the plant in the treatment of oxidative stress and toothache.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

Adesegun, S. A., Celestina, I. O. and Coker, H. A. (2016). Analgesic and Antioxidant Activities of Stem Bark Extract and Fractions of *Petersianthus macrocarpus. Pharmacognosy Res.* 8(3): 181–185.

Alan, L. and Miller N.D. (1996). Antioxidant Flavonoids: Structure, Function and Clinical Usage. *Alternative Medicine Rev*., 1: 103-111.

Alhassan M. A., Ibrahim M., and Musa I. A. (2014). Phytochemical Screening and Antimicrobial Evaluation of Stem Bark Extract of *Pseudocedrela kotschyi* (Herms). *British Journal of Pharmaceutical Research,* 4(16): 1937-1944.

Berhaut, J. (1971). Flore illustree du Senegal... dicotyledones: tome 1. Acanthaceesa Avicenniacees. *Dakar: Ministere du Developpement Rural 626p. Illustrations Geog*, *5*.

Bruce, A. (2006). Estimation of acute oral toxicity in rats by determination of the approximate lethal dose rather than the LD_{50}. *Journal of Applied Toxicology*, 6(1): 3-4.

Edeoga, H.O., Okwu, D.E. and Mbaebie, B.O. (2005). Phytochemical constituents of some Nigerian Medicinal plants. *African Journal of Biotechnology*, 4: 685-688.

Evan, W.C. (2009), *Trease and Evans Pharmacognosy*, Sixteen Edition Philadelphia, New York, London. PP 133-135

Ezeonwumelu J.O.C., Omar A.N., Ajayi A.M., Okoruwa A.G., Tanayen J.K, Kiplagat D.M. Okpanachi O.A., Abba S., Ezekiel I., Onchweri A.N., Okonkwo C.O. and Byarugaba F.(2012). Phytochemical screening, acute toxicity, anti-inflammatory and antipyretic studies of aqueous extract of the root of *Flueggea virosa* (Roxb. ex Willd.) in rats. *International Journal of Pharmaceutical and Biomedical Sciences;* 3(3): 128-135.

Feinberg S., Leong M., Bertagnolli A., Keller K., Pasero C., Fong A, and Feinberg

R. (2012). Resource Guide to Chronic Pain Medication and Treatment: Pain types and Chronic pain classification. *American Chronic Pain Association.* 2012 Edition. PP 8-11.

Ito, Y. (2005). Golden Rules and Pitfalls in Selecting Optimum Conditions for High-Speed Counter Current Chromatography. *Journal of Chromatography,* 1065, 145-168.

Jonathan, G. and Tom J. M. (2008).Secondary metabolites and the higher classification of angiosperms. Dept of Botany, Univ. of Texas, Austin, TX 78712, USA. *Nordic Journal of Botany 3(1):5 - 34.*

Khan H., Saeed M., Gilani A.U.H., Khan M.A., Dar A. and Khan I. (2010).The antinociceptive activity of *Polygonatum verticillatum* rhizomes in pain models. *Journal of Ethnopharmacology*; 127(2): 521–527.

Koster,R., Anderson, M. and Beer, E.J. (1959). Acetic acid for analgesic screening. Federation proceeds 18, PP 412-416.

Lakshman K., Shivprasad H.N., Jaiprakash B., and Mohan S. (2006). Anti-inflammatory and antipyretic activities of Hemidesmus indicus root extract. *African Journal of Traditional, Complementary and Alternative Medicines*; 3(1): 90-94.

Lorke D. (1983). A New Approach to Practical Acute Toxicity Testing. *Archives of Toxicology*; 54: 275-287.

Middleton, E., Drzewiecki, G., Krishnarao, D. and Kandaswami, C. (2000). Effect of Flavonoids on immune and inflammatory cell function. Advances Research Since 1986 (Harbone IR ed) Chapman and Hall, London. pp 619-645.

Mishra D., Ghosh G.,, Kumar P. S., and Panda P. K. (2011). An Experimental Study of Analgesic Activity of Selective Cox-2 Inhibitor with Conventional NSAIDs. *Asian Journal of Pharmaceutical and Clinical Research,* 4(1): 974-978.

Njoku, P.C. and Akumufula, M.I. (2007).Phytochemical and nutrient evaluation of spondias mombi Leave. *Pakistani Journal of Nutrition,* 6(6):613-615.

Ogbonnia, S.O., Mbaka, G.O., Anyika, E.N., Emordi, J.E. and Nwakakwa, N. (2011). An evaluation of acute and subchronic toxicities of a Nigerian polyherbal tea remedy. *Pakistan Journal of Nutrition,* 10: 115-127.

Ojewole, J. A. (2004). Evaluation of the analgesic, anti-inflammatory and anti-diabetic properties of *Sclerocarya birrea* (A. Rich.) Hochst. stem-bark aqueous extract in mice and rats.*Phytotherapy Research,* 18(8): 601-608.

Ojewole, J. A., Mawoza, T., Chiwororo, W. D. and Owira, P. M. (2010).*Sclerocarya birrea* (A. Rich) Hochst.[‘Marula’](Anacardiaceae): a review of its phytochemistry, pharmacology and toxicology and its ethnomedicinal uses. *Phytotherapy Research,* 24(5): 633-639.

Okigbo R.N., Anuagasi C.L., and Amadi J.E. (2009). Advances in selected medicinal and aromatic plants indigenous to Africa. *Journal of Medicinal Plant Research*; 3(2): 086-095.

Okwu, D.E and Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. *African Journal of Biotechnology,* 5(4): 357-361.

Okwu, D.E. and Okwu, M.E. (2004).Chemical composition of *Spondias mombin* Linn. plant parts. *Journal of Sustain. Agricultural Environment,* 6(2): 140-147.

Ozsoy, N., Can, A., Yanardag, R., Akev, N. (2008). Antioxidant activity of *Smilax excelsa* leaf extracts. *Food Chem*., 110: 571-583.

Patra, J. K., Gouda, S., Sahoo, S. K. and Thatoi, H. N. (2012). Chromatographic separation

1HNMR analysis and bioautography screening of Methanol extract of *Excoecarciaagallocha L.* from Bhitarkanika, Orissa, India. *Asian Pacific Journal of Tropical Biomedicine*, 17: 339-345.

Russo, D., Kenny, O., Smyth, T. J., Milella, L., Hossain, M. B., Diop, M. S. and Brunton, N. P. (2013). Profiling of phytochemicals in tissues from *Sclerocarya birrea* by HPLC-MS and their link with antioxidant activity. *ISRN Chromatography*, 11????.

Salawu O.A., Chindo B. A., Tijani A.Y., Obidike I.C., Salawu T.A. and Akingbasote A.J. (2009). Acute and sub-acute toxicological evaluation of the methanolic stem bark extract of *Crossopteryx febrifuga* in rats. *African Journal of Pharmacy and Pharmacology* 3(12): 621-626.

Sani, U. and Dailami, S. A. (2015). Synthesis, characterization, antimicrobial and antioxidant studies of metal (II) complexes of schiff base derived from 2-hydroxy-1-naphthaldehyde and hydrazine monohydrate, *Chemsearch Journal*, 6(2): 35-41.

Silva, B.A., Ferreres, F., Malva, J.O. and Dias, A.C.P. (2005). Phytochemical and antioxidant characterization of *Hypericum perforatum* alcoholic extracts. *Food Chem.*, 90: 157-167.

Somachit M.N., and Shahid A.R. (2003). Antipyretic and analgesic activities of *Zingiber zerumbet* extracts. *Proceeding of Regional Symposium on Environmental and Natural Resources*; 1:692-697.

Sowunmi, L. I., and Afolayan, A. J. (2015). Phytochemical constituents and antioxidant properties of acetone extract of *Cleome gynandra* (L.) growing in the Eastern cape, South Africa. *African Journal of Traditional, Complementary and Alternative Medicines*, 12(3): 1-8

Spencer, J.P.E., Powers, K., Smith-Weller, T.et al. (2008). Food for thought: The role of Diet Flavonoids in enhancing Human memory, learning and Neuro-cognitive Performance. *Proc Nutr Soc* 67: 238-253.

Stannard C., Collett B., Myles J., Roberts K., Stevenson B and Williams A.C. (2007). Pain and problem drug use: Information for patients. *The British Society for Pain.* Churchill House 35 Red Lion Square London WC1R 4SG.

Stark T.D., Mtui D.J., and Balemba O.B. (2013). Ethnopharmacological Survey of Plants Used in the Traditional Treatment of Gastrointestinal Pain, Inflammation and Diarrhoea in Africa: Future Perspectives for Integration into Modern Medicine. *Animals;* 3:158-227.

Stary, F. (1998). The Natural Guide to Medicinal Herbs and Plants. Tiger Books International, London pp. 12-16.

Ugbogu, E A., Emmanuel, O., Iheanyichukwu E, Friday U., Emmanuel A., Chinyere G. C, Faith, E. and Chizoba J. U (2016). Toxicological assessment of the aqueous dried leaf extracts of *Senna alata* L. in wistar rats. *African Journal of Pharmacy and Pharmacology*,10 (3): 709.

Varalakshmi, K.N., Sangeetha, C.G., Samee, U.S., Irum, G., Lakshmi, H., Prachi, S.P. (2011).In vitro safety assessment of the effect of the five medicinal plants on human peripheral lymphocytes, *Tropical Journal of Pharmaceutical Research*, 10: 33-36.

Walum E. (1998). Acute oral toxicity. *Environmental Health Perspectives* 106: 497- 503.