Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2014; 2 (6): 213-218
Received: 10-02-2014
Accepted: 25-02-2014

Mensah A.Y.
Department of Pharmacognosy, Faculty of Pharmacy & Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
Email: aymensah@yahoo.com;
Tel: +233-262-884544

Mireku E.A.
Department of Pharmacognosy, Faculty of Pharmacy & Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
Email: emireku@yahoo.com
Tel: +233-246-471364

Mensah M.L.K.
Department of Herbal Medicine, Faculty of Pharmacy & Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
Email: mlkmensah@yahoo.com
Tel: +233-208-968340

Amponsah I.K.
Department of Pharmacognosy, Faculty of Pharmacy & Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
Email: ik_amponsah@yahoo.com
Tel: +233243888337

Correspondence:
Professor Abraham Yeboah Mensah
Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
Email: aymensah.pharm@knust.edu.gh
Tel: + 233 03220 60366

Some neurological effects of the ethanolic stem bark extract of *Cussonia bancoensis* Aubrev and Pellgr (Araliaceae)

Mensah A.Y., Mireku E.A., Mensah M.L.K., Amponsah I.K.

ABSTRACT

In this study, some neurological effects of the 70% ethanolic stem bark extract (CB) of *Cussonia bancoensis*, Aubrev and Pellgr, (Araliaceae) were investigated in mice using various models. The effect of CB on the central nervous system (CNS) was studied using changes in general behavioural profiles (Irwin's test), tail flick test, potentiation of pentobarbitone induced sleep and effect on convulsion threshold in mice. Administration of doses between 100 and 3000 mg/kg body weight showed marked reduction in spontaneous activity, decreased response to touch and pain, moderate loss of balance, presence of ptosis and sedation. There was no mortality recorded after 24 and 48 h of administration of a maximum dose of 3000 mg/kg body weight. The study has shown that the stem bark extract at doses between 300 and 3000 mg/kg body weight has a dose -dependent sedative effect. This was observed as a reduction of locomotor activity and marked potentiation of pentobarbitone induced sleep onset and duration in mice. The ethanolic extract of *C. bancoensis* (CB) also significantly increased latency to tail withdrawal at 54 ±1 ºC in a dose dependent manner. Effect of CB extract on the onset and duration of pentylenetetrazole (PTZ) induced seizures was however insignificant. Based on these results, the traditional use of *C. bancoensis* in the management of pain and its ability to induce dizziness and sedation are justified. This observation may be attributed to the presence of plant secondary metabolites such as saponins and tripterpenoids which tested positive in the preliminary phytochemical screening of the powdered stem bark.

Keywords: *Cussonia bancoensis*, Hypnotic, Spontaneous Activity, Pentobarbital Sleeping time, Analgesia.

1. Introduction

A wide variety of traditional uses ranging from the treatment of diseases to magical applications and making of implements are found in Africa for the genus *Cussonia*. The main medicinal uses of *Cussonia* are as analgesic, anti-malarial, anti-inflammatory, treatment for sexually transmitted, bacterial and fungal infections, mental illness and epilepsy [1]. *Cussonia bancoensis* is one of the 21 species of the genus *Cussonia*, native to Ghana, but also planted in Ivory Coast, Nigeria, Liberia and Cameroon [2]. In Ghana it is commonly known as "kwae boofre" and has traditionally been used in folkloric medicine for the management of chronic inflammatory diseases, pain and in wound healing [3-4]. A decoction of the bark powder is drunk for the treatment of lower back pain or lumbago [5]. The people of Berekum in Ghana take the root bark decoction with ginger and pepper orally for the treatment of sexually transmitted [6], gynecological and gastric infections [3]. In Nigerian folklore medicine, the stem bark decoction is known to cause dizziness and is used to treat infertility [7]. Previous phytochemical screening of *C. bancoensis* revealed that flavonoids and triterpene saponins were the main phyto-constituents present in the stem bark [8, 9]. Previous biological evaluation established the anti-inflammatory, peripheral anti-nociception, antioxidant, nitric oxide inhibition and anticancer properties of some isolated constituents from the stem bark extract of *C. bancoensis* [5, 9-12]. To the best of our knowledge however, studies on *Cussonia bancoensis* for any possible effect on the central nervous system (CNS) are not yet reported. Folkloric report indicates that the stem bark extract induces sleep implying a role on the CNS. On the basis of this, we investigated the activity of the ethanolic stem bark extract of *C. bancoensis* on general behavioural pattern, pain, pentobarbital sleeping time and effect on convulsive threshold in mice.

2. Materials and Methods

2.1 Collection

The stem bark of *C. bancoensis* was collected from Nkawkaw-Asakraka, (06º33'0"ºN, º0º46'0"ºW) in the Eastern region of Ghana, in September, 2013. The plant material was authenticated at the Department of Herbal Medicine, Faculty of Pharmacy, KNUST, Ghana and voucher specimen (No. KNUST/CB1/2013/S005) was deposited.

2.2 Plant processing and Extraction (Cold maceration)

The plant material was washed with water, dried at room temperature for 5 days and ground into coarse powder with a mechanical grinder. Two hundred grams (200 g) of the powdered plant material was soaked in 600 mL of 70% ethanol. The container was sealed and shaken occasionally for 48 h. The mixture was then filtered and the filtrate obtained (ethanolic extract) was concentrated using a rotary evaporator (R-114, Buchi, Switzerland) at 60 ºC under reduced pressure. This gave a dark brown gummy concentrate (extract) which was further dried on a water bath to give a dark brown solid mass weighing 10.05 g. The dried extract was kept in a desiccator till required for use.

2.3 Phytochemical Screening

Preliminary phytochemical screening was performed on the powdered stem bark of *C. bancoensis* using standard procedures [13] for the identification of plant secondary metabolites such as alkaloids, flavonoids, steroids, saponins, reducing sugars, terpenoids and tannins.

2.4 CNS activity screening

2.4.1 Experimental animals

Male Imprinting Control Region (ICR) mice weighing (20-25 g) obtained from Noguchi Memorial Institute for Medical Research, Accra, Ghana, were used in the experiment. The animals were maintained under standard environmental (relative humidity 50 ± 10 %, room temperature 25 ± 2 ºC and 12 h light and 12 h dark cycle) and nutritional conditions throughout the experiment. The animals were kept for one week for acclimatization to the laboratory environment before use. They were deprived of food 24 h before the experiment. All experimental protocols were in compliance with the National Institute of Health guidelines for the care and use of laboratory animals and were approved by the Department of Pharmacology, KNUST, Ethics Committee.

2.4.2 Standard drugs

The following drugs were used in the experiment: Diazepam, Pentobarbitone, Pentylenetetrazole (PTZ) and Caffeine were purchased from Sigma- Aldrich Inc., St. Louis MO, USA. Morphine hydrochloride was obtained from Phyto-Riker, Accra, Ghana.

2.4.3 Experimental design

Animals were randomly selected and put into groups of six animals each. The groups were the negative control, positive control and experimental or treatment groups. The negative control groups received distilled water which was the vehicle for reconstituting the extracts, the positive control received different doses of standard drugs and the experimental groups received the extract at different doses in milligram per kilogram bodyweight (mg/kg). The vehicle and the extracts were administered orally (*p.o.*) while the standard drugs were administered intraperitoneally (*i.p.*).

2.4.4 General behavioural changes and acute toxicity studies

The general behavioural profile test was carried out by a qualitative assessment procedure [14]. The animals were grouped into five (n=6). Group 1 was the negative control which received the vehicle (distilled water) whiles groups 2, 3, 4 and 5 received the ethanolic stem bark extract of *C. bancoensis* at 100, 300, 1000, 3000 mg/kg body weight. Animals were observed at 15, 30, 60, 90, 120 and 180 min after administration of drug and also at 24 and 48 h later. Parameters which were observed included death, convulsion, tremor, straub tail, aggression, sedation, excitation, motor in-coordination, loss of grasp, loss of traction, altered reactivity to touch, loss of righting reflex and altered response to pain among others.

For the toxicity study, the groups of mice were orally administered with different doses of the stem bark extract as above and the mortality was determined after 24 and 48 h.

2.4.5 Analgesic activity

Central analgesic activity was measured by the tail flick test [15]. The time taken for the mouse to withdraw its tail from hot water maintained at 54 ± 1 ºC was measured. A cut-off latency of 10 s was set to avoid tissue damage. Increase in tail withdrawal latency was the measure of anti-nociception. Animals were tested at single time points of 30 min and observed for 120 minutes after administration of CB extract orally (30, 100, 300 mg/kg, *p.o.*) and morphine intraperitoneally (1, 3, 10 mg/kg *i.p.*). The control group received distilled water orally (2 mL, *p.o.*). The maximum possible anti-nociceptive effect was calculated as:

$$\% \text{ Maximal Possible Effect (MPE)} = [(T_1-T_0)/(T_2-T_0)] \times 100$$

Where T_0 and T_1 are defined as the latencies obtained before and after drug treatment respectively, and T_2 is the cut-off latency.

2.4.6 Pentobarbitone Induced Sleep

ICR mice were randomly put in 6 groups (n = 6). The mice were treated with CB extract at doses of 300, 1000 and 3000 mg/kg, *p.o.*), diazepam (4 mg/kg, *i.p.*), caffeine (8 mg/kg, *i.p.*) and distilled water (2 mL, *p.o.*). Thirty minutes after administration of drug or extract, sodium pentobarbitone (50 mg/kg, *i.p.*) was administered to each mouse to induce sleep. Mice were observed for the latency to or onset of sleep (time between pentobarbitone administration to loss of righting reflex) and duration of sleep (time between loss and recovery of righting reflex) [16].

2.4.7 Convulsion Threshold Test (PTZ- Induced Seizures)

The mice were divided into 7 groups (n=6). Groups 1, 2 and 3 received CB extract orally at doses of 30, 100 and 300 mg/kg body weight; groups 4, 5 and 6 received diazepam intraperitoneally at doses of 0.3, 1 and 3 mg/kg body weight and the last group (control group) received distilled water orally (2 mL, *p.o.*). Seizures were induced with pentylenetetrazole (PTZ) 75 mg/kg, given sub-cutaneously (*s.c.*), 30 min after distilled water or diazepam and 1 h after CB extract. The activities of the mice were then video recorded and later observed for the frequency, duration and latency to tonic convulsions for 1 h [17-18].

2.48 Statistical Analysis

All the data obtained were expressed as mean ± standard error. Data was analyzed using one-way ANOVA followed by Newman Keuls' *post hoc t* test. The results obtained were compared to the negative control groups and considered significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$. All statistical tests were carried out using graph pad prism version 5 software.

3. Results

3.1 Phytochemical screening: The result of the phytochemical screening of the stem bark extract of *C. bancoensis* is summarized in Table 1 below.

Table 1: Phytochemical constituents present in the dried stem bark powdered of *C. bancoensis*

Phytochemical	Results
Condensed tannins	+
Reducing sugars (Glycosides)	+
Saponins	+
Alkaloids	-
Triterpenoids	+
Steroids	+
Flavonoids	+

+: present, -: not detected

3.2 General behavioural changes

The ethanolic stem bark extract of *C. bancoensis* in doses up to 3000 mg/kg body weight did not cause any mortality in mice during the 24 and 48 h period of observation after oral administration. It was observed throughout a 3 hour period that animals treated with CB extract (100, 300, 1000 and 3000 mg/kg) showed significant reduction in spontaneous activity, decrease in locomotor activity, reduced reactivity to touch and pain and were noticeably quiet lasting for about 90 minutes. A summary of the result is shown in Table 2.

Table 2: Effect of *C. bancoensis* stem bark extract in the Irwin's test

Treatment drug (mg/kg)	Sedation	Reactivity to touch	Perception of pain	Muscle tone	Loss of balance	Ptosis
CB 100	-	-	↓	-	-	-
CB 300	+	↓	↓	-	-	-
CB 1000	++	↓	↓↓	↓	+	-
CB 3000	++	↓↓	↓↓↓	↓↓	++	+

N = 6; - absent, += slight, ++= moderate, +++= marked. ↓= slight decrease, ↓↓= moderate decrease, ↓↓↓= marked decrease

3.3 Analgesic activity

Analgesic activity was investigated using the tail flick test in mice. Results are presented in figures 1a to 1d. The control group which did not receive any drug had the lowest latency to tail withdrawal and an increased perception to pain. Pretreatment with CB extract (30, 100, 300 mg/kg) caused a non-dose dependent increase in threshold for pain perception, resulting in an increased latency to tail withdrawal at all doses when compared to the control group. The percentage maximum analgesic effect for the extract was reached at 120 min and was given by CB 30 mg/kg body weight (fig 1b).

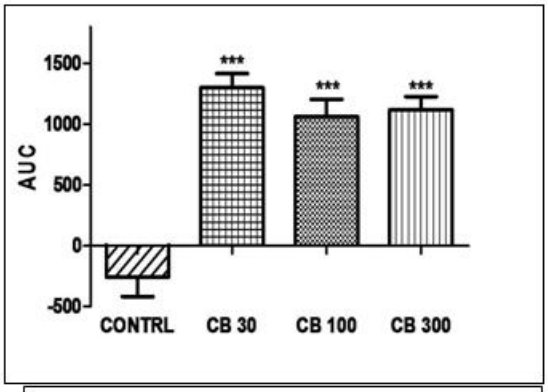

Fig 1a Effect of CB extract on tail withdrawal latency in mice when immersed in water at 54 ± 1ºC

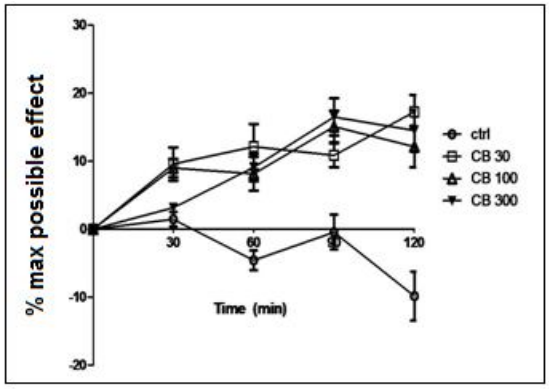

Fig 1b Time course curve showing the % maximal effect versus time for CB extract on tail withdrawal latency

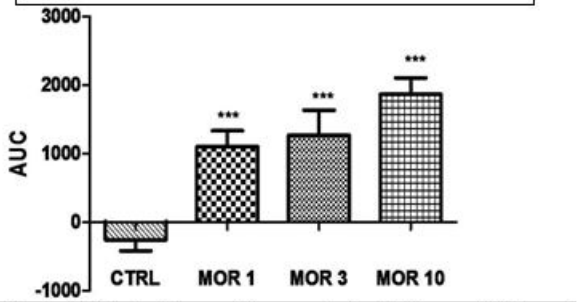

Fig 1c Effect of morphine on tail withdrawal latency in mice when immersed in water at 54 ±1 ºC

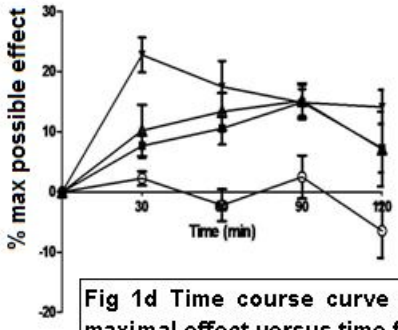

Fig 1d Time course curve showing the % maximal effect versus time for morphine on tail withdrawal latency

3.4 Pentobarbitone induced sleep

Prior administration of CB extract (300, 100, 3000 mg/kg) significantly potentiated pentobarbitone–induced sleeping time in mice. Various sleep time of mice treated with pentobarbitone with or without extract are shown in Table 3. CB extracts potentiated pentobarbitone induced sleep in a dose dependent manner when compared to control which received no drug. The normal sleep duration given by control group who received only pentobarbitone was 105 min while maximum sleep duration of 204 min was given by CB extract at 3000 mg/kg. Prior administration of CB extract at 3000 mg/kg also reduced the time of sleep onset in mice (3.8 min), closely following that of the standard drug diazepam 4 mg kg- which gave a sleep onset at 3.5 min.

Table 3: Effects of CB extract on pentobarbitone induced sleep in mice

Treatment drug	Dose	Onset of sleep	Duration of sleep
Distilled water	2 mL	5.630± 0.4735	105.2±2.709
CB	300mg/kg	5.148±0.9633	159.4±16.65**
CB	1000 mg/kg	4.535±0.779	177±22.25*
CB	3000 mg/kg	3.894±0.2049	204±23.96*
Diazepam	4 mg/kg	3.537±0.1681	284±12.53***
Caffeine	8 mg/kg	6.076±0.5083	80.93±16.64

Values are mean ± SME; n = 6 in each group; *significantly different at P<0.05. **significantly different at P<0.01, *** significantly different at P<0.0001

3.5 Convulsive threshold test

From the results, there was no significant effect of CB extract (30, 100 and 300 mg/kg) on the latency and duration of pentylenetetrazole (PTZ) 75 mg/kg induced convulsions. The standard drug, diazepam (0.3, 1 and 3 mg/kg), however, produced a dose dependent delay in the onset of tonic convulsions as well as a significant reduction in the duration of tonic convulsion in mice.

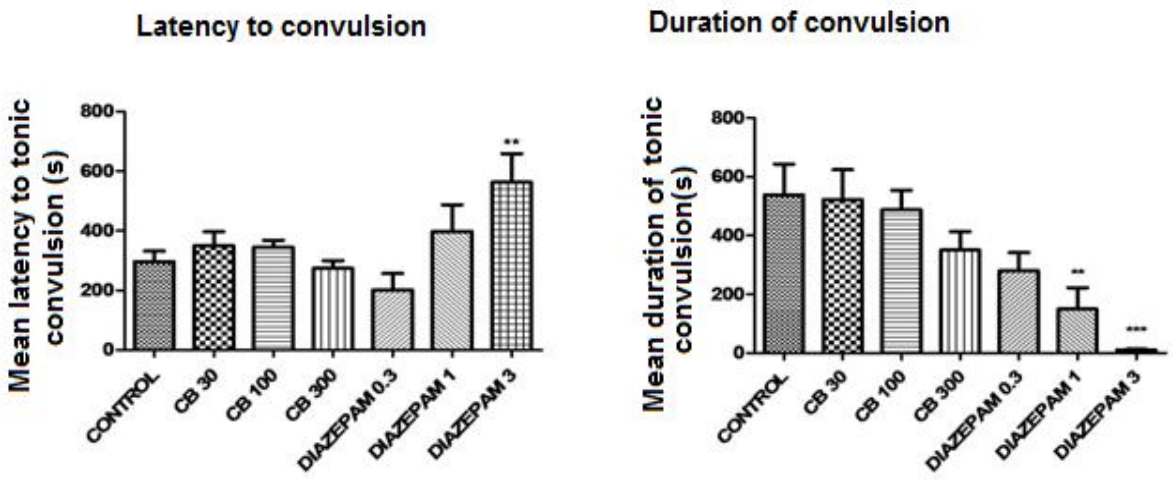

Fig 2a : Latency to and duration of PTZ-induced tonic convulsions after treatment with CB extracts at 30, 100 and 300 mg/ kg and diazepam 0.3, 1 and 3 mg/kg;significantly different at P<0.01, *** significantly different at P<0.0001**

4. Discussion

The stem bark extract of *C. bancoensis* is known for its ability to induce dizziness and sedation. The present study provided some data on the effect of the ethanolic stem bark extract on the central nervous system with regards to general behavioural changes, central analgesia, sedation or hypnotic effect and anti-convulsing properties in mice models. Preliminary phytochemical screening of the bark powder revealed the presence of condensed tannins, free reducing sugars, saponins, triterpenoids, plant steroids and flavonoids. This result is consistent with previous works which stated that the main phytochemical constituents of the stem bark of *C. bancoensis* are triterpene saponins, flavonoids and glucose [8, 10].

The tail flick test is usually considered appropriate for centrally acting analgesic drugs. Tail withdrawal latency was defined as the time (in seconds) taken to withdraw the tail from hot water maintained at 54 ±1.0 °C. Results from tail flick test indicates that the stem bark extract possess a strong non- dose dependent analgesic effect which was comparable to morphine at different doses. Previous works by Tapondjou *et al* (2003) [9] and Shin *et al* (2004) [5] reported on the peripheral analgesic effect of the stem bark of *C. bancoensis* and concluded that two triterpenes, ursolic acid and its derivative 23- hydroxy ursolic acid are responsible for the peripheral analgesic effect of the stem bark extract. The result of the tail flick test in this study however suggests a centrally mediated analgesic effect of the extract. The efficacy of most herbal remedies may be attributed to several secondary metabolites which act by different mechanisms to produce their effects. It can therefore be said that the extract's analgesic effect may be both peripherally and centrally mediated.

The sedative effect of drugs is generally evaluated by measurement of spontaneous motor activity and pentobarbitone induced sleeping time in laboratory animal models [19]. From the results, *C. bancoensis* stem bark extract produced a significant alteration in general behavioural pattern of animals as shown in the Irwin's test. This was observed as a reduction in locomotor activity, decreased spontaneous activity, general quietness and moderate sedation which lasted for about 90 minutes. In addition to the changes in gross behavioral pattern, *C. bancoensis* extract at different doses produced a significant potentiation of the hypnotic effect of pentobarbitone in a dose dependent manner. This suggests that *C. bancoensis* possess a marked sedative effect which is as a result of the depressant action on the central nervous system [20]. The behavioural pattern changes obtained from this results are thus in accordance with the folkloric use of *C. bancoensis* suggesting a sedative or hypnotic activity.

The CNS depressant action of some plant secondary metabolites such as triterpenoids and saponins has been indicated by several scientific reports [21-23]. It is reported that some saponins show a potent sedative activity and reduced spontaneous activity when tested in similar mice models [24]. The presence of saponins and triterpenoids in this plant may therefore be contributing to the observed CNS depressant activity of the stem bark extract of *C. bancoensis*.

5. Conclusion

The results of this study justify the folkloric knowledge of *Cussonia bancoensis*' ability to induce sedation and gives credence to its use in the management of pain.

6. References

1. De Villiers BJ, Van Vuuren SF, Van Zyl RL, Van Wyk BE. Antimicrobial and antimalarial activity of Cussonia species (Araliaceae). Journal of Ethnopharmacology. 2010; 129(2):189-196.
2. Irvine FR. Woody plants of Ghana. Woody plants of Ghana. 1961.
3. De Villiers B, Tilney P, Van Wyk B. The taxonomic significance of leaf anatomical characters in Cussonia and related genera (Araliaceae). Botanical Journal of the Linnean Society. 2010; 164(3):246-263.
4. Burkill H. The useful plants of west tropical Africa. Edition 2. Vol. 1: families AD1985.
5. Shin K-M, Kim R-K, Azefack TL, David L, Luc SB, Choudhary MI, et al. In vitro anti-inflammatory activity of 23-hydroxyursolic acid isolated from *Cussonia bancoensis* in murine macrophage RAW 264.7 cells. Planta medica. 2004; 70(09):803-807.

6. Effah KB. Illness and curing in Berekum, Ghana: Theses (Dept. of Sociology and Anthropology)-/Simon Fraser University; 1991.
7. Adjanohoun E, Ahyi MR, Ake-Assi L, Elewude JA, Dramane K, Fadoju SO, et al. Contribution to Ethnobotanical Floristic Studies in Western Nigeria, Lagos,Nigeria: Organization of African Unity, Scientific Technical and Research Commission 1991.
8. Haruna A, Ilyas M, Mustapha A. Triterpene-saponin from the stem bark of *Cussonia bancoensis* Aurev and Pellegr. Ghana Journal of Chemistry. 1994; 1(9):401-403.
9. Tapondjou LA, Lontsi D, Sondengam B.L , Choi J., Lee K.T., Jung HJ, et al. In Vivo anti-nociceptive and anti-inflammatory effect of the two triterpenes, ursolic acid and 23-hydroxyursolic acid, from *Cussonia bancoensis*. Archives of pharmacal research. 2003; 26(2):143-146.
10. Tapondjou L, Talat M, Iqbal CM, Lontsi D, Sondengam LB, Atta U. Bancoensone, a new antioxidant caffeoyl derivative from *Cussonia bancoensis*. Cameroon Journal of Experimental Biology. 2006; 1(1):57-61.
11. Takaya M, Nomura M, Takahashi T, Kondo Y, Lee K-T, Kobayashi S. 23-hydroxyursolic acid causes cell growth-inhibition by inducing caspase-dependent apoptosis in human cervical squamous carcinoma HeLa cells. Anticancer research. 2009; 29(4):995-1000.
12. Tapondjou LA, Lontsi D, Sondengam BL, Shaheen F, Choudhary MI, Atta-Ur-Rahman H, et al. Saponins from *Cussonia bancoensis* and their inhibitory effects on nitric oxide production. Journal of Natural Products. 2003; 66:1266-1269.
13. Harborne JB. Phytochemical methods A Guide to modern techniques of plant analysis: springer; 1998.
14. Irwin S. Comprehensive behavioral assessment: A systematic quantitative procedure for assessing the behavioral and physiologic state of the mouse. Psychopharmacologia 1968; 13:222-257.
15. Steinmiller CL, Young AM. Pharmacological selectivity of CTAP in a warm water tail-withdrawal antinociception assay in rats. Psychopharmacology. 2008; 195(4):497-507.
16. Paton WD, Pertwee R. Effect of cannabis and certain of its constituents on pentobarbitone sleeping time and phenazone metabolism. British journal of pharmacology. 1972; 44(2):250-261.
17. Löscher W. Animal models of epilepsy for the development of antiepileptogenic and disease-modifying drugs. A comparison of the pharmacology of kindling and post-status epilepticus models of temporal lobe epilepsy. Epilepsy research. 2002; 50(1):105-123.
18. White HS. Clinical significance of animal seizure models and mechanism of action studies of potential antiepileptic drugs. Epilepsia. 1997; 38(s1):S9-S17.
19. Lu M-C. Studies on the sedative effect of *Cistanche deserticola*. Journal of ethnopharmacology. 1998; 59(3):161-165.
20. Fujimori H, Cobb, D. Potentiation of barbital hypnosis as an evaluation method for central nervous system depressant. Psychopharmacology. 1995; 7:374-377.
21. Chattopadhyay D, Arunachalam G, Mandal SC, Bhadra R, Mandal AB. CNS activity of the methanol extract of *Mallotus peltatus* (Geist) Muell Arg. leaf: an ethnomedicine of Onge. Journal of ethnopharmacology. 2003; 85(1):99-105.
22. Subarnas A, Tadano T, Oshima Y, Kisara K, Ohizumi Y. Pharmacological Properties of β-Amyrin Palmitate, a Novel Centrally Acting Compound, Isolated from *Lobelia inflata* Leaves. Journal of pharmacy and pharmacology. 1993; 45(6):545-550.
23. Srikanth J, Muralidharan P. CNS activity of the methanol extracts of Sapindus emarginatus Vahl in experimental animal models. Journal of Scientific Research. 2009; 1(3):583-593.
24. Dubois M, Ilyas M, Wagner H. Cussonosides A and B, two triterpene-saponins from *Cussonia barteri*. Planta medica. 1986; 52(02):80-83.