AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 43 NUMBER 1 MARCH 2014

ISSN 1116–4077

Afr. J. Med. med. Sci. (2014) 43, 11-16

Central nervous system activity of the ethanol leaf extract of *Sida acuta in rats*

GF Ibironke[1], AS Umukoro[2] and DC Ajonijebu[1]
Departments of Physiology[1] and Pharmacology and Therapeutics[2], College of Medicine, University of Ibadan, Ibadan, Nigeria.

Abstract

Background: The study investigated the pharmacological effects of ethanol extract of *Sida acuta* leaves on central nervous system activities in mice.

Methods: Adult male mice (18 - 25g) were used for the study. The extract was administered orally in male mice and evaluated in the following tests: forced swimming, tail suspension, formalin-induced paw licking, acetic acid – induced mouse writhing and apomorphine-induced stereotypy.

Results: The results revealed a reduction in the frequency of abdominal constrictions induced by acetic acid, decreased licking times in both phases of the formalin test, reduction in immobility times in forced swimming and tail suspension tests. However, the extract produced no effect on apomorphine-induced stereotyped behaviour.

Conclusion: These results suggest that the ethanol extract of *Sida acuta* contains psychoactive substances with analgesic and antidepressant-like properties which may be beneficial in the management of pain.

Keywords: *Sida acuta; pain; depression; sedative.*

Résumé

Introduction: L'étude consistait à enquêter sur les effets pharmacologiques d'extrait d'éthanol des feuilles du*Sida acuta* sur les activités de système nerveux central chez les souris.

Méthodes: Les souris mâles adultes (18 - 25g) ont été utilisées pour l'étude. L'extrait a été administré oralement chez les souris males, et évalué dans les épreuves suivantes: la natation forcée, la suspension de queue, la raclée de patte incitée de formol, la souris incite, se tortillant et apomorphine-incitée stéréotype a l'acide acétique

Résultats: Les résultats ont révélé une réduction de la fréquence de constrictions abdominales incitées par l'acide acétique, les temps de raclée diminués dans les deux phases de l'épreuve de formol, la réduction aux temps d'immobilité dans la natation forcée et les épreuves de suspension de queue. Pourtant, l'extrait n'a produit aucun effet sur le comportement stéréotypé apomorphine-incité.

Conclusion: Ces résultats suggèrent que l'extrait d'éthanol du*Sida acuta* contient de substances psychoactive avec les propriétés analgésiques et semblables à des propriétés d'antidépresseur qui peuvent être favorables dans la gestion de douleur.

Correspondence: Dr. G.F. Ibironke, Department of Physiology, College of Medicine, University of Ibadan, Ibadan Nigeria.
E-mail: gibironk@yahoo.com

Introduction

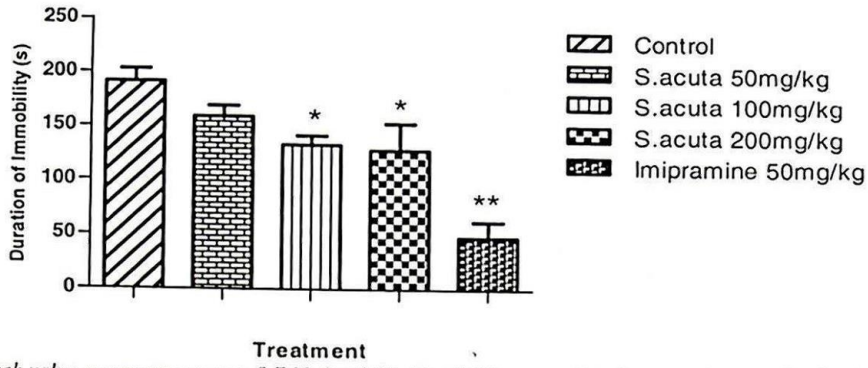
Over the years, statistics indicate that out of approximately 45 million people suffering from mental or behavioural disorders, only a small minority could access basic treatment. This may progressively compound a global burden of psychiatric disease, proposed to rise to 15% by 2020 [1]. This led to a progressive research on medicinal plants to constantly demonstrate the psychotherapeutic effectiveness of different plant species in varieties of animal models [2,3] for the treatment of neurological disorders.

Sida acuta is a shrub belonging to the Malvaceae family [4] and commonly referred to as teaweed, ironweed [5]. Locally, it is called osonkotu/ isankotu [6] or iseketu among the Yoruba tribe in Nigeria. The plant is an erect annual and/or perennial shrub that can grow to a height of three feet. The stems are woody, branching several times, and it has a well-developed tap root. The leaves are lance-to rhomboid-shaped with serrated margins and have small yellow flowers that can be solitary or growing in pairs in the leaf axils [7].

It is widely distributed in the tropics and subtropical regions [8] and known to frequently dominate improved pastures, waste dumps and road sides [4].

Sida acuta is tonic, astringent and emollient. The plant is useful in the treatment of blood, throat, respiratory and urinary tract related infections and pain including fever and headache (9). Decoction of the leaves had also been used for the treatment of hookworm, diarrhea, parasitic skin diseases, catarrh, dysentery and nephritis [10].

Several phytochemical screenings resulted in isolation of alkaloids and steroidal compounds [11, 12]. The main alkaloids are cryptolepine and its derivatives such as quindoline, quindolinone, cryptolepinone and 11-methoxy-quindoline. The major steroids are ecysterone, beta-sistosterol,



*Each value represents mean ± S.E.M, *p<0.05, **p<0.01compared to the control group; Student's t-test (n = 6).*
Fig. 2. Effects of ethanol extract of *sida acuta* leaves on immobility duration in mice in tail suspension test.

Table 1: Effect of ethanol extract of *sida acuta* on formalin induced paw licking in mice.

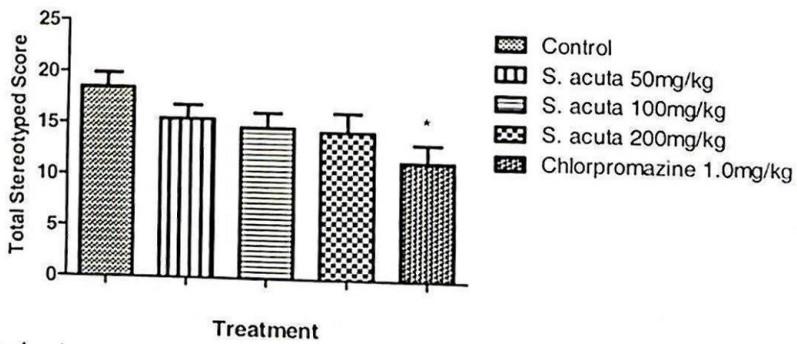
Treatment	Dose (mg/kg)	1st Phase Licking Times (s)	% Inhibition	2nd Phase Licking Times (s)	% Inhibition
Control	-	80.67 ± 5.94	-	146.50 ± 17.72	-
S. acuta	50	62.00 ± 8.03	23.14%	75.67 ± 13.58**	48.35%
S. acuta	100	58.67 ± 4.25*	27.27%	53.17 ± 9.34***	63.71%
S. acuta	200	53.00 ± 5.72**	34.30%	41.50 ± 11.09***	71.67%
Indomethacin	50	50.50 ± 5.84**	37.40%	69.00 ± 28.42*	52.90%

*Each value represents mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001 compared to the control group; Student's t-test (n = 6).*

Table 2: Effects of ethanol extract of *sida acuta* leaves on acetic-acid induced writhing in mice.

Treatment	Dose (mg/kg)	Writhing number/ 30mins	% Inhibition
Control	-	112.20 ± 7.92	-
S. acuta	50	80.40 ± 10.51*	28.34%
S. acuta	100	63.60 ± 4.06***	43.32%
S. acuta	200	59.40 ± 7.32**	47.32%
Acetylsalicyclic Acid	100	61.80 ± 12.09**	44.92%

*Each value represents mean ± S.E.M. *p<0.05, **p<0.01, ***p<0.001 compared to the control group; Student's t-test (n = 6).*



*Each value represents mean ± S.E.M. *p<0.05 compared to the control group; Student's t-test (n = 6).*
Fig. 3: Effects of ethanol extract of *sida acuta* leaves on apomorphine-induced stereotyped behaviour in mice.

Acetic acid – Induced Writhing Test

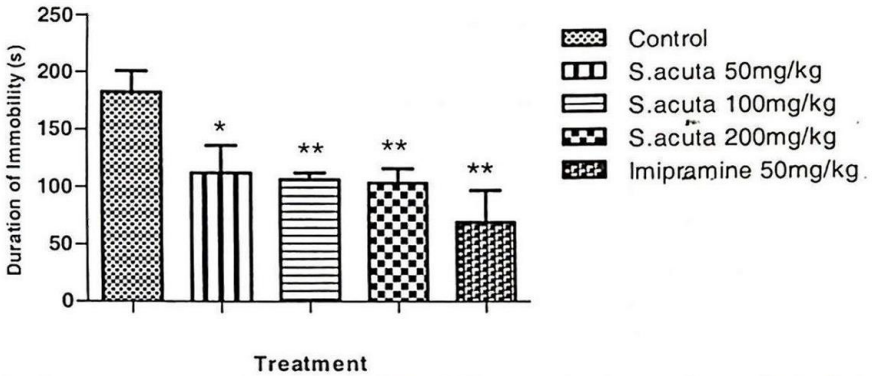
The effect of *Sida acuta* on acetic acid-induced nociception in mice was assessed according to Konster *et al* [18]. Thirty minutes before intraperitoneal injection of 0.60% acetic acid to induce abdominal constrictions, the animals were pretreated with doses of *Sida acuta* (50–200 mg/kg, per oral (p.o.)), acetylsalicylic acid (100 mg/kg, p.o.) or distilled water (10 ml/kg, p.o.). After abdominal constrictions have been induced, each mouse is immediately transferred to an observation chamber and the number of abdominal constrictions (writhings) was measured for 30 minutes.

t-test; p values less than 0.05 were considered statistically significant.

Results

Effect of ethanol extract of sida acuta on immobility duration in mice in the forced swim test

Sida acuta (50 – 200mg/kg) significantly ($p < 0.05$) decreased the duration of immobility in the FST in a dose-related manner when compared with the control. The period of immobility was also significantly ($p < 0.05$) reduced by imipramine (50mg/kg).



Each value represents mean ± S.E.M, *p<0.05, **p<0.01 compared to the control group; Student's t-test (n = 6)

Fig. 1: Effects of ethanol extract of *sida acuta* leaves on immobility duration in mice in forced swim test.

Test for psychosis

Apomorphine – induced stereotypy test

A modified method of Bourin *et al* [19] was adopted for this stereotyped behavioural disorder as an animal model for psychosis. The animals were pretreated with *Sida acuta* (50–200 mg/kg, p.o.), chlorpromazine (1 mg/kg, p.o) or distilled water (10 ml/kg, p.o.) thirty minutes before injection of apomorphine (1.5 mg/kg, s.c.). Each mouse was observed in a transparent tank (21 x 16 x 14 cm) for 2 minutes at 10, 20, 30 and 45 minutes after subcutaneous administration of apomorphine. Stereotyped behaviours were scored as: 0, absence of stereotyped behaviour; 1, presence of stereotyped movements of the head; 2, intermittent sniffing; 3, chewing; and 4, intense licking [19]. The results were expressed as the sum of the individual stereotype score at each time interval in each group.

Statistical analysis

Results were expressed as mean ± standard error of mean (SEM). The data were analyzed using Student's

Effect of ethanol extract of sida acuta on immobility duration in mice in the tail suspension test

The extract of *Sida acuta* (50 – 200mg/kg) shortened the duration of immobility in mice in a dose-related manner in TST. Reduced duration of immobility caused by the extract was significant ($p<0.05$) only at doses of 100 and 200mg/kg of *Sida acuta* when compared with the control. Also, imipramine (50mg/kg) significantly ($p<0.05$) shortened immobility duration in mice.

Effect of ethanol extract of sida acuta on formalin-induced paw licking test

Mouse characteristic paw-licking response to formalin induced nociception occurred in two phases. Only doses of 100 and 200mg/kg of *Sida acuta* significantly ($p<0.05$) inhibited paw licking during the 1st phase when compared with the control. During the 2nd phase, *Sida acuta* produced a dose dependent and significant ($p<0.05$) inhibition of nociceptive responses in all tested doses (50 – 200mg/kg).

6. Odukoya O.A., Inya-Agha S.I and Ilori O.O. Immune boosting herbs: Lipid Peroxidation in Liver homogenate as index of activity. *Journal Pharm. and Toxicology.* 2007; 2(2): 190- 195.
7. Murphy T R , Colvin D L, Dickens R, *et al.* Weeds of southern turfgrasses. University of Florida, Cooperative Extension Service and Institute of Food and Agricultural Sciences Publication SP. 1996; 79: 208 p.
8. Holm LG, Plucknett DL and Pancho JV, Herberger JP. The World's Worst Weeds: distribution and biology- University Press of Hawaii, Honolulu, USA. 1977
9. Kerharo J and Adam JG. La pharmacopée sénégalaise traditionnelle: Plantes médicinales et toxiques. Ed Vigot frères Paris. 1974; 2 - 7114 – 0646 - 6.
10. Burkill HM. The Useful Plants West Tropical Africa. Royal Botanic Gardens. Kew. England. 1997
11. Cao JH and Qi YP. Studies on the chemical constituents of the herb huanghuaren (Sida acuta Burm. f.) Zhongguo Zhong Yao Za Zhi. 1993; 18: 681-2, 703.
12. Dinan L, Bourne P and Whiting P. Phytoecdysteroid profiles in seeds of Sida spp. (Malvaceae). Phytochem. Anal. 2001; 12: 110-119.
13. Jang DS, Park EJ, Kang YH, Su BN, Hawthorne ME, Vigo JS, Graham JG, Cabieses F, Fong HH, Mehta RG, Pezzuto JM, Kinghorn AD. Compounds obtained from Sida acuta with the potential to induce quinone reductase and to inhibit 7,12- dimethylbenz[a]anthracene-induced preneoplastic lesions in a mouse mammary organ culture model. Arch. Pharm. Res. 2003; 26 (8): 585-590.
14. Abdulrahman F, Inyang SI, Abbah J, *et al.* Effect of aqueous leaf extracts of Irvingia gabonesis on gastrointestinal tracts of rodents. India J. Exp. Biol. 2004; 42:787-791.
15. Porsolt RD, Anton G, Blavet N and Jalfre M. Behavioural despair in rats: A new model sensitive to antidepressant treatments. Eur J Pharmacol. 1978; 47: 379–391
16. Steru L, Chermatm R, Thierry B and Simon P. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacol. 1985; 85: 367 – 370.
17. Hunskaar S and Hole K. The formalin test in mice: Dissociation between inflammatory and non- inflammatory pain. 1987; 30: 103 – 114.
18. Konster R, Anderson M and Debeer EJ. Acetic acid analgesic screening. Fed Proc. 1959; 18: 418–420
19. Bourin M, Poisson L and Larousse C. Piracetam interaction with neuroleptics in psychopharmacological tests. Neuropsychobiology. 1986; 19:93–96.
20. Hunskaar S, Fasmer OB and Hole K. The formalin test in mice: A useful technique for evaluating mild analgesics. J Neurosci. Meth. 1985; 14: 69 – 76.
21. Gene RM, Segura L, Adzet T, Marin E and Iglesia J. Heterotheca inuloids: anti – inflammatory and analgesic effect. J Ethnopharmacol. 1998; 60: 157 – 160.
22. Miranda *et al.* Anti nociceptive and anti edematogenic properties and acute toxicity of tabebuia avellandae lonie griseb; Inner bark aqueous extract.B.M.C Pharmacol. 2001; 1:6
23. Tjolsen A, Berge O, Hunskaar S, Rosland JH and Hole K. The formalin test: an evaluation of the method. Pain. 1992; 51: 5 – 14.
24. Aladeokin AC and Umukoro S. Psychopharmacological properties of an aqueous extract of Tetracarpidium conophorum in mice. J Nat Med. 2011; 65:411–416.
25. Subarnas A, Tadano T, Nakahata N, *et al.* Possible mechanism of antidepressant activity of beta-amyrin palmitate isolated from Lobelia inflata leaves in the forced swimming test. Life Sci. 1993; 52:289–296.
26. Lucki I. The forced swimming test as a model for core and component behavioural effects of antidepressant drugs. Behav Pharmacol. 1997; 8:523–532.
27. Chindo BA, Amos S, Odutola AA, *et al.* Central nervous system activity of the methanol extract of *Ficus platyphylla* stem bark. J. Ethnopharmacol. 2003; 85: 131- 137.
28. Tarsy D and Baldessarini RJ. Movement disorders induced by psychotherapeutic agents: Clinical features, pathophysiology, and management. *In*: Shah NS, Donald AG (ed) Movement disorders, Plenum Press, New York. 1986; pp. 365-389.
29. Stolk JM and Rech RH. Antagonism of *O*-amphetamine by methyl-*p*tyrosin. Behavioural evidence for participation of cathelcolamine stores and synthesis in the amphetamine stimulant response. Neuropharmacology. 1970; 9: 249 – 263.

Effect of ethanol extract of sida acuta on acetic acid induced writhing in mice

The extract of *Sida acuta* (50 – 200mg/kg) produced a dose dependent inhibition of abdominal constrictions in mice. A greater inhibition (47.32%) was observed at a dose of 200mg/kg of the extract when compared with 44.92% inhibition produced by acetylsalicyclic acid (100mg/kg).

Effect of ethanol extract of sida acuta on apomorphine - induced stereotyped behaviour in mice

Sida acuta at all tested doses (50 – 200mg/kg) did not produce any significant ($p>0.05$) alterations in the behavioural patterns of the animals.

Chlorpromazine (1.0mg/kg), being the reference drug, significantly ($p<0.05$) suppressed the stereotyped behaviour induced by apomorphine.

Discussion

This study was carried out to investigate the central nervous system activity of the ethanol extract of *Sida acuta* in laboratory animals. Formalin-induced paw licking response [20] and acetic acid-induced mouse writhing tests [18] were employed as models for evaluating the analgesic properties of *Sida acuta*. This study showed that *Sida acuta* has an analgesic property, due to the ability of this plant extract to reduce the number of writhings or abdominal constrictions induced by acetic acid in mice. In rodents, the behavioural pain produced by acetic acid, a chemical irritant producing tissue necrosis and encoding tissue injury, was reported to be inhibited by several classes of analgesics [21].

Inhibition of nociceptive action of acetic acid by *Sida acuta* suggests the presence of phytochemically active substances in the extract. Formalin produced two phasic behavioural pains in rodents; the 1st phase (0 – 5minutes) represents a centrally mediated neurogenic pain which results from direct stimulation of primary afferent pain fibers and the 2nd phase (15 – 30minutes) is an inflammatory pain response [22, 17] which is peripherally mediated and it is due to activation of nociceptors by chemical mediators [23]. The demonstration that *Sida acuta* suppressed the biphasic pain response in mice is an indication of its strong analgesic property.

The two experimental models used for assessing the antidepressant – like property of *Sida acuta* were tail suspension test (TST) and forced swim test (FST). Both FST and TST evoked characteristic behavioural immobility in mice, following exposure to stressful experimental sessions such as swimming and body tail suspension respectively. Tiredness, lowered mood (hopelessness), reduced stamina and cessations of persisted escape – directed behaviour are all features of immobility [24] which represent some of the core symptoms seen in clinically depressed patients [25]. Through these tests, reduction of immobility periods in rodents by antidepressant drugs like imipramine have been shown to clinically correlate to a similar effect on mood elevation in depressed patients [26]. The outcome of the study showed that *Sida acuta* exhibited antidepressant – like activity due to its ability to reduce the period of immobility in mice in both FST and TST.

Drugs that inhibit apomorphine – induced stereotyped behaviour are known to antagonize dopamine receptors in the nigrostriatal region [27, 28]. In mice, apomorphine increases the intensity and duration of stereotypic behaviour, such as head movement, persistent sniffing, intense licking and chewing, by acting directly on the post-synaptic dopamine D_2 receptors [29]. Inhibition of these receptors thus reverses the hyperactivity and stereotypy suggesting interference with the central dopaminergic neurotransmission. In this study we observed that *Sida acuta* extract did not significantly alter the behavioural patterns induced by apomorphine thereby suggesting non interference with the central dopaminergic transmission..

It can be concluded based on these findings that *Sida acuta* contains psychoactive compounds with analgesic and anti-depressant-like properties without significant effects on the dopaminergic pathway.

References

1. Reynolds E.H. Brain and mind: a challenge for WHO. Lancet. 2003; 361, 1924-1925.
2. Onasanwo S.A., Chatterjee M. and Palit G. Antidepressant and anxiolytic potentials of Dichloromethane fraction from Hedranthera barteri. Afr. J. Biomed. Res. 2010; 13:1
3. Zhang ZJ. Therapeutic effects of herbal extracts and constituents in animal models of psychiatric disorders. Life Sci. 2004; 75: 1659 – 1699.
4. Mann A, Gbate M, and Umar AN. Sida acuta subspecie acuta. Medicinal and economic palnt of Nupeland, Jube Evans Books and Publication. 2003; p. 241.
5. Rick W, Tim B and Pat M. A native growing season forage for wildlife – Teaweed, Sida acuta Burm.f. Institute of food and agricultural sciences, University of Florida, Milton. FOR. 2011; 114: 1 – 4.

Introduction

High blood pressure causes one in every eight deaths worldwide, making it the third leading global cause of mortality [1]. In the year 2000 about one billion adults had hypertension all over the world, and the number is expected to rise to 1.56 billion in 2025 [2]. Elevated blood pressure (BP) is one of the risk factors for the development of cardiovascular diseases and may be exacerbated by regular consumption of caffeine [3]. Caffeine is one of the active biological components in coffee, tea, kolanut and other beverages [4]. More than 80% of the world's population ingest caffeine daily, making it the most widely consumed drug in history [5].

The genus *Cola* (Sterculiaceae) is comprised of about forty species, but the most commonly used are *Cola. anomala, C. acuminata, and C. nitida*, with the latter two being the most widely consumed. *Cola nitida* has been found to have the highest amounts of caffeine. However, despite the caffeine contents of kolanut, the number of studies on the health benefits of kolanut is negligible compared to coffee.

During the last decade, reports have been focused on effects of coffee and caffeine on diabetes mellitus [6 -8], neurological diseases [9], different types of cancer [10], hormonal changes [11-13], gallstones [14], renal stones [15], as well as cardiovascular disease [16], and hypertension [17].

Kolanuts have been used in folk medicine as an aphrodisiac and an appetite suppressant [19]. Previous studies indicate that chronic consumption of crude extract of kolanut caused tachycardia which was associated with increase in arterial blood pressure in normotensive and salt-induced hypertensive rats [19,20]. Intravenous injection of crude extract of kolanut increased the rate and force of contraction of isolated rat heart [21]. The precise mechanism of the hypertensive action of the kolanut crude extract could however not be established in these studies. Therefore, the aim of the present study is to investigate the vascular mechanisms of chronic consumption of kolanut extract involved in its hypertensive effect in rats by assessing the relaxation response of isolated aortic rings to acetylcholine (ACh) and sodium nitroprusside (SNP) in the presence or absence of L-nitroarginine methyl ester (L-NAME), Atropine and indomethacin.

Materials and methods

The experimental protocol for this study was approved by the Animal Research Ethics Committee of the Lagos State University College of Medicine, Nigeria. This is in conformational with the 1985 guidelines for laboratory animal care of the National Institute of Health (NIH).

Chemicals and drugs

Noradrenaline, ACh, SNP, L-NAME, indomethacin and atropine were obtained from Sigma–Aldrich (St. Louis, Mo., USA), while caffeine was extracted from kolanut. The drugs were diluted with distilled water and were freshly prepared on the day of the experiment.

Plant materials

Seeds of specie *Cola nitida* were used in this study. The seeds were purchased from a market in Lagos, Nigeria. Identification of the plant was carried out by the Taxonomist of the Forestry Research Institute. Following identification, a specimen voucher number FHI 1008881 of the plant was deposited in the herbarium of the Forestry Research Institute, Ibadan, Nigeria.

Preparation of kolanut crude extract

The seeds were air dried under shade for two weeks and thereafter macerated at room temperature to powdered form. One kilogram of the powdered seeds was obtained and exhaustively extracted with ethanol. Powdered kolanut was extracted (1kg per 2 litres) two times with ethanol and water (80:20 V/V) for 1 h at room temperature, the solvent was evaporated at 40°C under vacuum and final ethanolic extract lypholized. The extract solution was prepared as a suspension with 4g /100mL of saline as the stock solution.

Extraction of caffeine from kolanuts

Methylene chloride (Dichloromethane-DCM) was used for the isolation of caffeine from kolanut briefly describe as follows: a known weight of dried and ground samples (Caffeine products) was extracted three successive times with hot water (100°C) and cold water in a dark place (Flask covered with aluminium foil) at room temperature (25°C). The collected extracts were filtered using filter paper, the filtrate was evaporated to eliminate the solvent using rotary evaporator (at 45°C), and the obtained residues (crude extracts) were kept in the refrigerator until use [22]. Then 100 mL of the kolanut extract was added to a clean 500 mL Erlenmeyer flask, and mixed with two grams of sodium bicarbonate ($NaHCO_3$), another 25 mL of (DCM) was added and the mixture vigorously swirled for 10 minutes. This was allowed to mix till two separate layers were formed a dark aqueous top layer and a clear methylene chloride bottom layer. The upper layer contained the caffeine while the bottom layer was the decaffeinated. All of