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EFFECT OF AQUEOUS LEAF EXTRACT OF *COMBRETUM MICRANTHUM* G. DON (*COMBRETACEAE*) ON GASTRO INTESTINAL SMOOTH MUSCLE

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ABSTRACT
The effects of the aqueous leaf extract of Combretum micranthum were studied on gastro intestinal smooth muscle of rodents. The extract was screened using isolated rabbit jejunum, guinea pig ileum and rat uterus. The extract produced relaxation of isolated rabbit jejunum and guinea pig ileum. The relaxation of guinea pig ileum was inhibited by phentolamine. The effect of the extract on rabbit jejunum and guinea pig ileum may involve adrenergic receptors. The extract had no effect on pregnant and non-pregnant isolated rat uterus. The result of preliminary phytochemical screening of the extract showed that, the aqueous leaf extract contains alkaloids, flavonoids, glycosides, saponins, tannins and phlabotannins. The properties of the extract may be due to the presence of these active constituents of pharmacological importance that bear relevance to its therapeutic claims in traditional medicine.
Keywords: Combretum micranthum, Rabbit jejunum, Rat uterus and Guinea pig ileum.

INTRODUCTION

Plants have always been a common source of medicaments, either in the form of traditional preparations or as a pure active principle. Most drugs of plant origin used by medical practitioners are in form of extract of the part or whole plant for example digoxin from *Digitalis lanata* (Trease and Evans, 1989) and the discovery of tubocurarine from *Chondodendronto mentosun* (Hans, 1996).

Combretum micranthum is locally known as *farargeza* (Hausa), *Okan* (Yoruba) and *Nzaotego* (Igbo) and belongs to the family of *Combretaceae.* It is widely distributed in savannah regions and in some places near the coast as a shrub or a tree and found in Northern part of Nigeria, where it is used for the treatment of diarrhoea, skin disease and to stop bleeding (Burkill, 1985). *Combretum micranthum* has a pleasant taste and appreciated as a digestive aid (Burkill, 1985). *Combretum micranthum,* is of great medicinal importance, used in the treatment of diarrhoea and various skin diseases. Personal communication with herbalist showed that, leaf of *C. micranthum* is used in the management of diarrhoea, blood clotting, pain and tumor. Forty years ago, a plague in the Gambia locally named "Alibain", claimed many lives but a lot of people were cured using hot tea of *C. micranthum* leaves (Laxen, 2001). The leaves of *C. micranthum* are used traditionally as cough syrup, treatment of malaria, fever and all ailments of the liver and gall bladder (Trease and Evans, 1996). The leaves of *C. micranthum* and *C. racernosum* are used as herbal remedy for the treatment of diarrhoea and various skin diseases (Kolaand Benjamin, 2002).An aqueous extract of the fresh leaves of *C. micranthum* was reported to contain antibacterial agent (Udum *et al.,* 2012).Some species of *Combretaceae* family have been investigated and have been revealed to possess a large number of bioactive compounds, which exert strong antioxidant and antimicrobial activities (Karou, *et al.,* 2005). *Combretaceae* family is rich in tannins, saponins, sterols, carbohydrates, glycoside and trace of alkaloid (Trease and Evans, 1997). The objective of this study was to investigate the phytochemical constituents and the effect of aqueous leaf extract of *Combretum micranthum* on isolated rabbit jejunum, guinea pig ileum and rat uterus.

MATERIALS AND METHODS

Plant collection and extraction

Fresh leaves of *Combretum micranthum* were collected from Malumfashi L.G.A, Katsina State. Plant was identified and authenticated in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria, by comparing with voucher specimen number 900257. Leaves were air dried under the shade at room temperature (30^{o}C) for 28 days and then ground into a fine powder using pestle and mortar. About 700 g of powdered material was soaked in water for two weeks and maceration method was used in the extraction. The extract was concentrated on water bath at temperature of 60^{o}C.

Animals

Rats (weighing150 – 180g), a rabbit (weighing 2.0 - 2.5 kg) and guinea pigs (weighing 250 – 300g) of either sex were used for the experiments. Animals were obtained from animal House of Faculty of Pharmaceutical sciences, Ahmadu Bello University Zaria. Animals were kept in a well-ventilated room, fed with a pelletized grower mash (vital) and water provided *ad-libitum*. Rabbit and Guinea pig were starved for 24 hours before the experiment.

Preliminary Phytochemical screening
Preliminary screening of the aqueous leaf extract of *Combretummicranthum* was conducted using standard methods of Harbone (1989) and Trease and Evans (1997).

Isolated Tissue Studies
Method of Amos *etal.* (2000) was used. An overnight starved rabbit (weighing 2.0 – 2.5kg) and an adult guinea pig (weighing 300 – 400g) which had free access to water were sacrificed by a knock on the head, exsanguinated and the abdomen opened. Segment of the rabbit jejunum and guinea pig ileum of about 2 to 3cmlong each were removed and mounted in a 25 ml organ bath containing Tyrode's solution at 37 ^{0}C and aerated with air. Thirty minutes equilibration period was allowed and the physiological solution was changed every 15 minutes. At the end of the equilibration period, the effects of extract and acetylcholineon the rabbit jejunum were evaluated. The effectof the extract on the guinea pig ileum was investigated, while phentolamine and propranolol were used as antagonists on guinea pig ileum. The doses used for theextract (mg/ml), acetylcholine ($\times10^{-8}$mg/ml), phentolamine ($\times10^{-7}$mg/ml) and propranolol ($\times10^{-7}$mg/ml) are 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4. The contact time for each concentration was 1minute while time cycles were 3 minutes. The responses were recorded usingUgo Basile microdynamometer 7050 with speed of 24 mm/min and sensitivity of 2.

Isolated rat uterus
The experiment was based on the method of Vongtau *et al.*(2000). Female pregnant and non-pregnant rats were used. Non pregnant rats were pretreated with stilbestrol 0.1mg/kg (*i.p.*), 24 hours before the experiments were carried out in order to bring the rats to oestrus stage. The animals were killed and the abdomen cut open to reveal the uterine horns. The two horns were separated and transferred into a Petri dish containing De Jalon's solution. The tissues were suspended in 25 ml organ bath containing De Jalon's solution at 37^{0}C and aerated with air. One end of the tissue was tied to the hook of the aerator and the other end to a transducer. The tissue was equilibrated for 30minutes with the physiological solution changed every 10minutes. At the end of the equilibration period, the effects of extract andoxytocin on the rat uterus were investigated.The doses used for both extract (mg/ml) and oxytocin ($\times10^{-5}$mg/ml) are; 0.4, 0.8, 1.6, 3.2 and 6.4. The contact time for each concentration was 1minute while time cycles are 3 minutes. The responses were recorded on microdynamometer 7050 with speed of 24mm/min and sensitivity of 2.

RESULTS
Preliminary phytochemical study
The preliminary phytochemical screening of the extract revealed the presence of valuable constituents as showed in Table 1. Alkaloids, flavonoids, glycosides, saponins, tannins and phlabotannins were present while sterols and terpenes were absent.

Effects of aqueous leaf extract of *C. micranthum* and acetylcholine on isolated rabbit jejunum
The relaxation effects of aqueous leaf extract of *C. micranthum* and contraction activities of acetylcholine on isolated rabbit jejunum are presented in Figure 1. Acetylcholine produced a dose- dependent contraction (Fig. 1a) while the extract produced a dose-dependent relaxation of rabbit jejunum (Fig. 1b). The relaxation effect of the extract produced no effect on acetylcholine (Ach) induced contraction of rabbit jejunum (Fig. 1c).

Effects of aqueous leaf extract of *C. micranthum* ,phentolamine and propranololon isolated guinea pig ileum
The activities of extract, phentolamine and propranolol on isolated guinea pig ileum were presented in Figure 2. The aqueous leaf extract of *C. micranthum* produced a dose-dependent relaxation effect on isolated guinea pig ileum (Fig. 2a). The relaxation effect produced by the extract on guinea pig ileum was inhibited by phentolamine but unabolished by propranolol (Figures 2b and 2c).

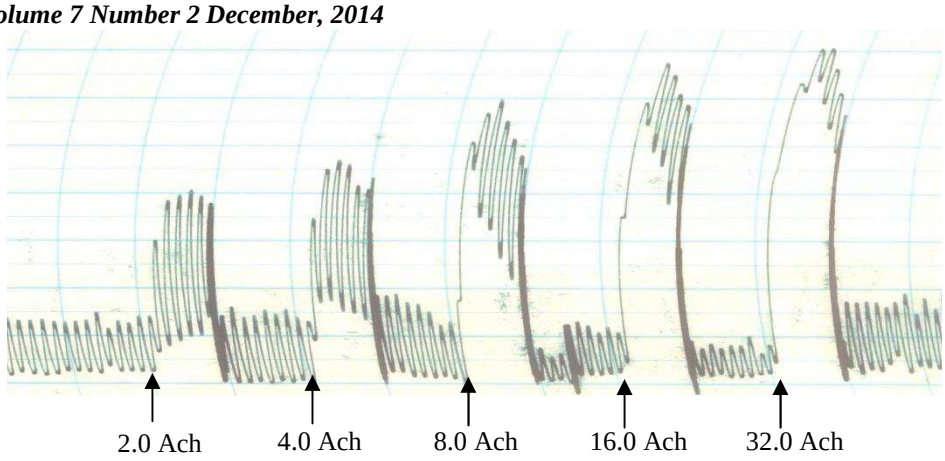
Effect of oxytocin and aqueous leaf extractof *C. micranthum*on pregnant and non-pregnant isolated rat Uterus
The effect of oxytocin and aqueous leaf extract of *C. micranthum* on pregnant and non-pregnant isolated rat uterus are showed in Figure 3. The extract showed no response on both pregnant and non-pregnant rat uterus but oxytocin produced contraction on both uterus (Figures 3a and 3b).

Table 1: Preliminary phytochemical screening of aqueous leaf extract of *C. micranthum*

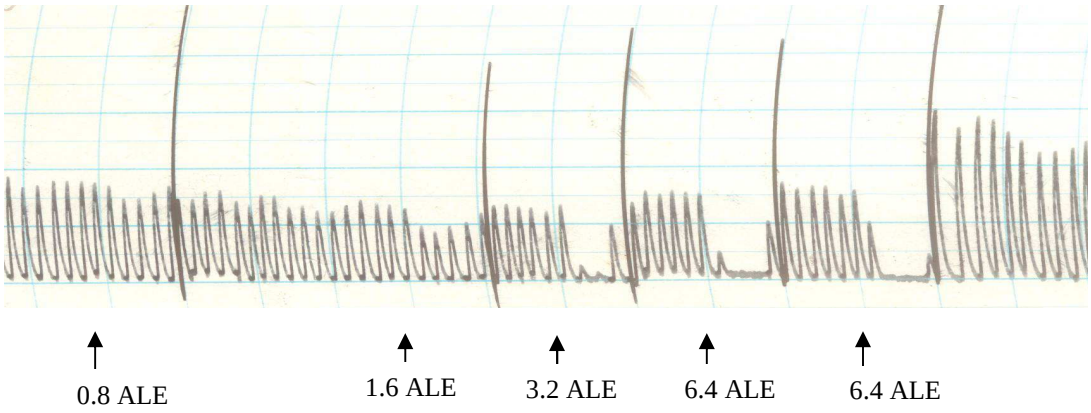
Constituents	Inference
Alkaloids	+
Flavonoids	+
Glycosides	+
Saponins	+
Sterols	-
Tannins	+
Terpenes	-
Phlabotannins	+

Key: + Presence, Absence

a.



b.



c.

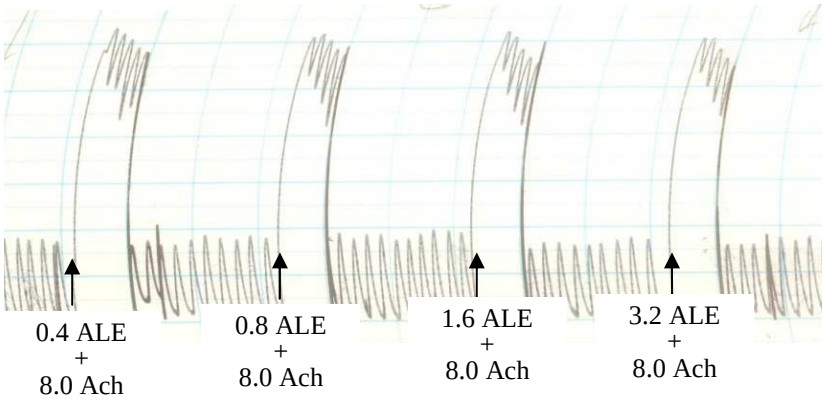
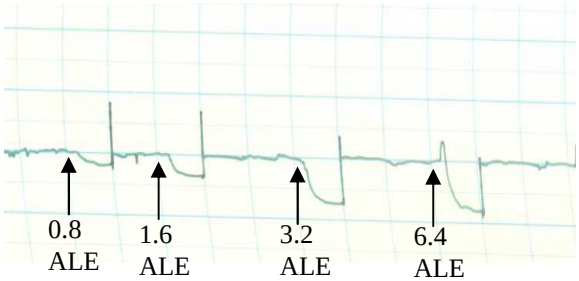


Fig. 1: The relaxation and contraction effects of aqueous leaf extract of *C. micranthum* (mg/ml), and acetylcholine ($\times 10^{-7}$ mg/ml) on rabbit jejunum, ALE= Aqueous LeafExtract and Ach= Acetylcholine.

a.



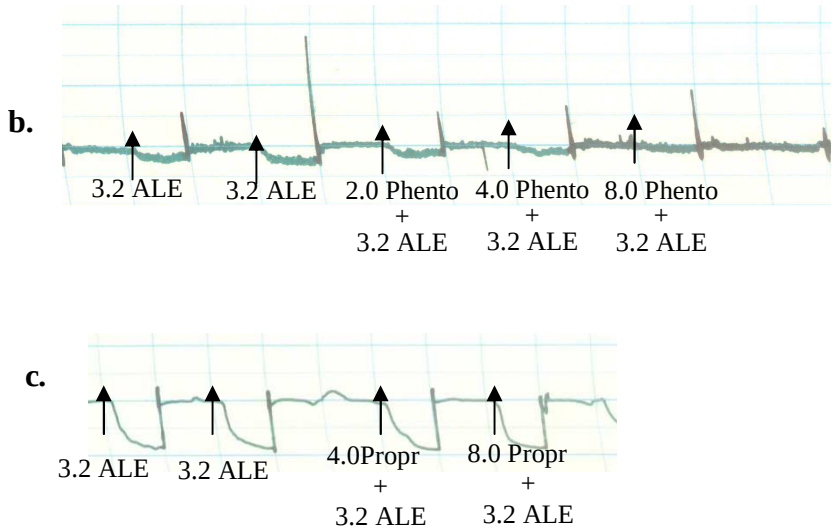


Fig. 2: The effect of phentolamine (×10^{-6} mg/ml) and propranolol (x10^{-6}mg/ml) on relaxation of guinea pig ileum induced by aqueous leaf extract of *C. micranthum* (mg/ml). Phento = Phentolamine, Propr. = Propranolol, and ALE = Aqueous Leaf Extract.

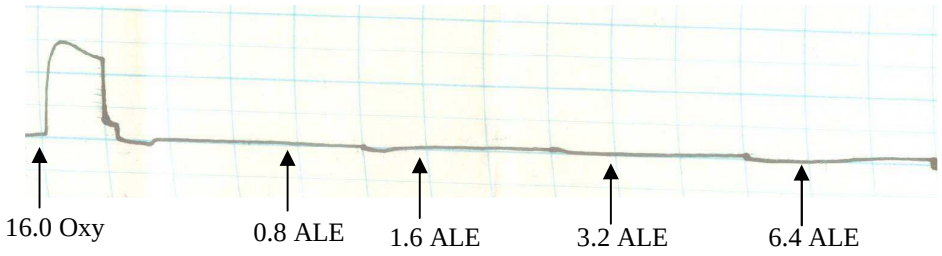


Fig. 3a: The effect of aqueous leaf extract of C. *micranthum* (mg/ml) and oxytocin (×10^{-4} mg/ml) on non-pregnant rat uterus, ALE= Aqueous Leaf Extract and Oxy = Oxytocin.

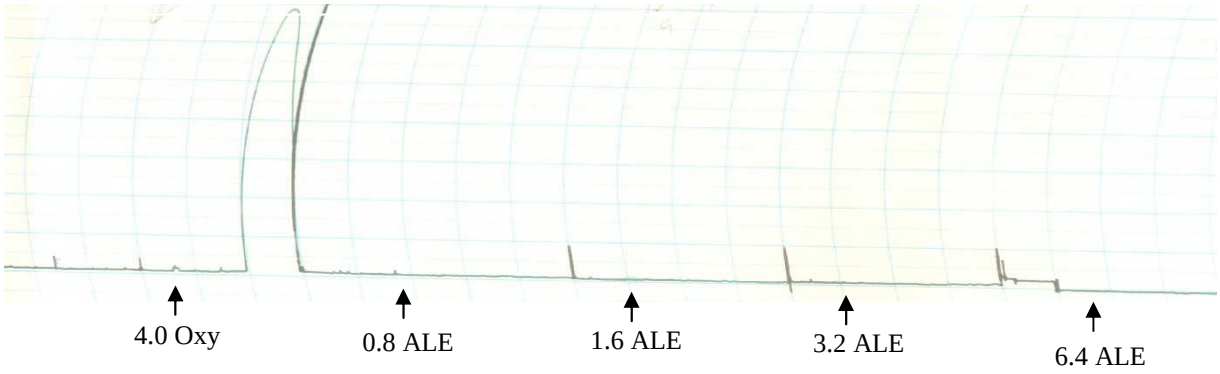


Fig. 3b: The effect of aqueous leaf extract of *C. micranthum* (mg/ml) and oxytocin (×10^{-4} mg/ml) on pregnant rat uterus, ALE = Aqueous Leaf Extract and Oxy = Oxytocin.

DISCUSSION

The present study on the aqueous leaf extract of *C.micranthum* revealed the presence of chemical constituents which can induce relaxation of rabbit jejunum and guinea pig ileum. The preliminary phytochemical screening conducted on the aqueous leaf extract of *C.micranthum*showed that the leaf contained valuable constituents which are of great therapeutic importance. Some chemical constituents such as alkaloids, flavonoids, saponins and tanninsfound present in the extract are known to have various pharmacological activities on man and animals. The same authors had also reported that the aqueous leaf extract is less-toxic via*i.p* administration in mice and rats and can be used for folkloric medicine (Abdullahi *et al.,* 2014). The extract produced relaxation of isolated rabbit jejunum and guinea pig ileum, but the relaxation effect on rabbit jejunum did not antagonize contractions induced by acetylcholine.

Acetylcholine induced contraction of smooth muscle results from activation of muscarinic receptors and differences in muscarinic receptor are now known to exist (Bonner, 1989). The relaxant effect of the extract on guinea pig ileum was antagonized by phentolamine while propranolol had no effect on both rabbit jejunum and guinea pig ileum (Fig. 3). Propranolol is a nonselective β- receptor blocking agent. The extract may have α-adrenergic activity, since the relaxation effect on guinea pig ileum was inhibited by phentolanime, a non-selective α-receptor antagonist. Phentolamine antagonist postsynaptic α_1-receptors and presynaptic α_2 –receptors of smooth muscle and one of the adverse reactions of phentolamine is gastrointestinal disturbance as a result of hypersecretion (David, 2001). The relaxation effect of the extract on gastrointestinal smooth muscle may reduce gastrointestinal tract motility. The extract may produce its effect through α_2-adrenoceptor because the jejunum and ileum have α_2, β_2–adrenoceptors while uterus has α_1, β_2 – adrenocepters (David, 2001). The relaxation effect on rabbit jejunum and guinea pig ileum were not affected by acetylcholine and propranolol respectively. Oxytocin produced contractile response on pregnant and non-pregnant rat uterus, but the extract showed no response on pregnant and non- pregnant rat uterus. Hence, the result of this study suggests that aqueous leaf extract of *C. micranthum* could be considered safe to use in traditional medicine by pregnant and non-pregnant women. The adrenergic activity of the extract may be due to the presence of tannins and flavonoids and found to be responsible for the plant activity as claim by local herbalist. Several investigators have indeed attributed the antidiarrhoeal and anti-inflammatory properties of a number of plants to their flavonoids constituent (Oyewole, 2003). Flavonoids and tannins isolated from *Scleroarya birrea* bark and *quercitrin* were studied in rats and mice respectively and they were found to be involved in the anti-diarrhoea activity (Galvez *etal*., 1991; Galvez *etal*., 1993).

CONCLUSION

The aqueous leaf extract of *C. micranthum* contains pharmacological active principle(s), which may account for the beneficial effect of the plant in the management of diarrhoea as claim by traditional medicine.

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