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PHYTOCHEMICAL AND ANALGESIC EVALUATION OF METHANOL LEAF EXTRACT OF *Clerodendrum volubile* Linn.

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ABSTRACT

The toxic effects of known analgesics in pains and stress management are major health concern globally. This study therefore investigated the phytochemical and analgesic property of commonly used medicinal plant in southwestern Nigeria for pain management. *Clerodendrum volubile* (CVL) was screened for phytochemical constituents following standard procedure. Forty two Wistar Albino Mice (WAM) of both sexes (35-40 g, n=6) were grouped into seven. Thirty minutes prior to intraperitoneal injection with 2 ml of 0.1% acetic acid, animals in groups I- III received plant extracts (1 mg/kg, 10 mg/kg, 100 mg/kg respectively), IV- VI were treated with paracetamol (acetaminophen), aspirin and indomethacin while VII received saline water. Thirty minutes observation period was adhered to. Time related analgesic effect was also investigated. The results showed that the extract contained alkaloids, tannins, saponins, phenols, anthraquinones, and flavonoids. Analgesic assay revealed methanol leaf extract of CVL demonstrated a dose dependent activity on the acetic acid-induced writhing. Writhing counts significantly reduced in mice administered with 1-100 mg/kg CVL (85.17±3.72, 57.17 ± 4.07 and 40.00 ± 2.08) compared to paracetamol, aspirin, indomethacin (76.50±7.64, 45.67± 2.73, 33.50 ± 4.23) and saline water (207.83±9.95). Percentage inhibition of abdominal constriction was dose dependent in methanol leaf extract of CVL (87.00±0.03%) relative to control (0.00%). These findings support the previously reported analgesic and anti-inflammatory activities of extracts of other species related to the study plant.

Keywords: *Clerodendrum volubile*, medicinal plants, musculoskeletal disorder, phytochemical.metals.

INTRODUCTION

Musculo-skeletal pain is one of the most common reasons for which people seek medical attention, thus analgesics are among the commonly prescribed medications in clinical practices (Benedicta *et al*., 2010). However, the more common analgesics are falling out of favour because of their toxic side effects on chronic use; the non-steroidal anti-inflammatory drugs (NSAIDs) cause gastric ulcerations while acetaminophen causes liver damage (Bower *et al*., 2007). Consequently, it is important to search for analgesics which have less adverse side effect.

The genus *Clerodendrum* is widely distributed in the tropics and sub-tropics. It comprises of small trees, shrubs and herbs (Shrivastava and Patel, 2007, Erukainure *et al*., 2014). *Clerodendrum* is known to be a very large and diverse genus with over 580 identified species which are widely distributed in Asia, Australia and America (Erukainure *et al*., 2014). Among many species of Clerodendrum genus is *C. volubile*.

Clerodendrum volubile (magic leaf or white butterfly leaf) is commonly found in southern part of Nigeria. It is known for its food and potent medicinal values though there is absence of scientific database for this claims. *C. volubile* is a climbing shrub of about 3 m, glabrous except the inflorescences. It is found in the deciduous forests and secondary jungles (Burkill, 1985).

Clerodendrum volubile has been used traditionally in the treatment of ailments like arthritis, rheumatism, dropsy, swelling, oedema, gout, general healing, pain killers, pregnancy, anti-abortifacients, sedatives, etc. (Burkill, 1985, Erukainure *et al*., 2011). The leaves are often dried and used locally as spices in cooking and sometimes the fresh leaves are blended and used for cooking as soup concoction. Its use in the treatment and in management of several ailments made it to be referred to as magic leaf. However, those claims have not been scientifically proven (Erukainure, 2014).

The present study has been designed to unravel

the pharmacological basis for the use of *C. volubile* in the management of pain using animal models.

METHODS

Plant Collection

The leaves of *Clerodendrum volubile* (CVL) was collected during the rainy season (between April and August) in 2012 and the authentication of the plant was carried out at Forestry Research Institute (FRIN), Herbarium, Jericho, Ibadan, Oyo State, Nigeria, where the voucher specimen was kept and voucher Number (FHI - 108884) was allocated.

Phytochemical Screening

The leaves were cleaned, washed with water and dried under shade for a period of 14 days. The dried leaves were later milled into a fine powder with a blender. The fine powder was weighed and kept in a clean container for further use. The powdered plant sample was tested by standard phytochemical screening procedure for the presence of alkaloids, anthraquinones, flavonoids, tannins, saponins, terpenes, steroid, cardenolides and chalcones (Harbone (1973, Trease and Evans (1989) and Sofowora (1993).

Animals

Swiss albino mice of both sexes weighing between 22 – 25g were used for the study. The animals were maintained under standard environmental conditions and were fed with standard pellet diet and water *ad libitum*.

Analgesic activity

CVL crude extract at the doses of 1, 10, and 100 mg/kg were used. The activities of these doses were compared with the standard drugs: aspirin (100 mg/kg), paracetamol (100 mg/kg) and indomethacin (50 mg/kg) to determine the acetic-acid induced writhing response in mice. The extract concentrations and standard drugs were administered to seven groups of six mice per group.

Extract Preparation.

The leaves of *Clerodendrum volubile* were dried under shade for about a month and this was later ground to powder. 200 g of the powdered plant material was extracted with methanol continuously 72 hours with soxhlet extractor. The extract was then concentrated to dryness using rotary evaporator at reduced pressure and kept in desiccators pending analgesic assay.

Acetic acid – induced writhing test:

Two (2) ml of acetic acid solution (0.1%) was injected intraperitoneally (i.p) and the number of writhes during the following 30 minutes period was observed (Jam and Rao, 1987). Abdominal constriction (squirming syndrome) was selected as the only end point observation in this study.

Statistical analysis

The results obtained were subjected to statistical analysis using SPSS version 18. For objectivity and to remove bias, all measurements were done in replicates and standardized using Excel package for windows. Data were subjected to analysis of variance (ANOVA) and means were separated using Duncan Multiple Range Test (DMRT) at $p < 0.05$.

RESULTS

Table 1 showed the qualitative phytochemical constituents of *Clerodendrum volubile* crude methanol, water and diethyl-ether leaf extracts. The result revealed that methanol extract contained alkaloid and phenol in appreciable quantity, saponin, tannin and athraquinones in moderate amount while cardenolides and chalcones were absent. Most of the phyto-constituents were present in appreciable amounts in diethyl-ether except for cardenolides, chalcones and flavonoid available in trace quantity. Most of the phyto-constituents were absent in water extract except alkaloid, saponin, and phenol.

Table 1: Qualitative Phytochemical constituents of leaf extracts of *C. volubile*.

Constituents	Methanol	Water	Diethyl ether
Alkaloids	+++	++	+++
Saponins	++	+++	+++
Tannins	++	+	+++
Phlobatannins	+	+	++
Phenols	+++	++	+++
Anthraquinones	++	-	+++
Terpenes	+	-	++
Cardenolides	-	-	+
Steroids	+	-	++
Glycosides	+	++	+++
Chalcones	-	-	+
Flavonoids	+	-	+

Key:
+++ Present in an appreciable amount, ++ Present in a moderate amount
+ Present in minute amount
- Completely absent

Table 2 showed the effect of crude methanol leaf extract of *Clerodendrum volubile* on abdominal constrictions induced by acetic-acid in mice and the reaction time. *Clerodendrum volubile* significantly decreased the mean number of abdominal constriction or writhes, which was dose dependent. In addition, the methanol leaf extract increased the percentage inhibition of abdominal constriction from 0% in the negative control to 87.01% at highest concentration of 100 mg/kg, this effect was found to be superior to that of paracetamol and aspirin. In addition, when compared to the reference drugs paracetamol, aspirin and indomethacin, there was no significant difference in the reaction time.

Table 2: *Clerodendrum volubile* methanol leaf extract on abdominal constrictions and reaction time induced by acetic acid in mice.

Treatment	Dose (Mg/kg)	Writhing count Mean ± SD	% Inhibition	Reaction Time (mins) Mean ± SD
Control (saline water)	------	207.83 ± 9.95^{a}	-----	3.67 ±1.03^{c}
PCM	100	76.50 ± 7.63^{b}	63.19	9.33 ± 0.82^{a}
ASP	100	45.67 ± 2.73^{d}	78.02	9.67 ±1.03 ^{a}
IMC	50	33.50 ± 4.23^{e}	83.88	8.33 ±0.52^{a}
CVL1	1.0	85.17 ± 3.72^{b}	68.58	6.50 ± 0.34^{b}
CVL2	10	57.17 ± 4.07^{c}	77.78	8.00 ± 1.24^{ab}
CVL3	100	40.00 ± 2.08^{de}	87.01	9.00 ± 0.37 ^{a}

Values are represented as means ± SEM (n=6 readings) compared with the control by Duncan's Multiple Range Test (DMRT) at $p<0.05$. PCM= paracetamol, ASP= aspirin, IMC= indomethacin, CVL1 (1mg/kg), CVL2 (10 mg/kg) and CVL3 (100 mg/kg) represent groups of rats treated with *Clerodendrum volubile* methanolic leaf extracts.

Table 3 showed the comparison reaction time effect of *Clerodendrum volubile* methanol leaf extract on abdominal constrictions induced by acetic acid in mice. It was observed that, the inhibitory time increases as the induction time increased, this relationship was noticed at 30, 90, and 120 minutes.

Table 3: Time related analgesic effect of *Clerodendrum volubile* methanol leaf extract on abdominal constrictions induced by acetic acid in mice

Dose of Treatment	Writhing counts 30 mins.	Writhing counts 60 mins.	Writhing counts 90 mins.	Writhing counts 120 mins.
CVL1	85.17 ± 3.72^{a}	64.50 ± 1.29^{a}	32.17 ± 1.85^{a}	23.33 ± 2.74^{a}
CVL2	57.17 ± 4.07^{b}	32.67 ± 5.88^{b}	10.17 ± 1.35^{b}	12.00 ± 1.98^{b}
CVL3	40.00 ± 2.08^{c}	34.67 ± 1.71^{b}	3.00 ± 0.68^{c}	10.00 ± 0.86^{b}

Key: CVL1= *C. volubile* (1mg/kg); CVL2 = *C. volubile* (10mg/kg); CVL3 = *C. volubile* (100mg/kg). Values are Mean ± SD Means with the same letters are not significantly different at $p<0.05$

DISCUSSION AND CONCLUSION

Clerodendrum volubile water extract possessed alkaloids, saponins, tannins, phlobatannins, phenols and glycosides; this is almost similar to the report of Erukainure *et al.,* (2011). The methanol extract possessed alkaloids, saponins, tannins, phenols, anthraquinones, terpenes, phlobatannins, steroids, glycosides and flavonoids and the diethyl ether phyto-constituent was similar, thus the choice of methanol extract for the bioactivity assay. The alkaloids contents were higher than the result observed by Erukainure, (2011).These bioactive components which are known to be analgesic, bactericidal, pesticidal or fungicidal in nature may be responsible for the activity observed in the plant. Alkaloids viz- a - viz: morphine, cocaine, thiamine and cannabinol have been reported to contain analgesic activities in certain plants (Ogbeche *et al*., 2003). These alkaloids are used in the pharmaceutical industries in the production of analgesics due to their analgesic properties (Okwu and Ndu, 2006; Erukainure *et al.,* 2011). It has been established that acetic acid causes increase in peritoneal fluid levels of prostaglandins, consequently causing inflammatory pain by inducing capillary permeability (Amico-Roxas *et al*., 1984, Adedapo *et al*., 2013). The present study, as a result of the observed effects, suggests that *C. volubile* had an inhibitory potential on prostaglandin synthesis.

The analgesic potency of *C. volubile* was dose dependent, as their effects increased with increasing oral dose of the methanolic extracts. From the analgesic assay, it was observed that *C. volubile* possessed dose-dependent activity and that there was a significant decrease in the writhing counts when compared to paracetamol and control. In this study, the reference drugs (paracetamol, aspirin and indomethacin) gave a 63.19%, 78.02% and 83.88% inhibition of writhing in animals while 100 mg/kg CVL dose gave 87.01% inhibition indicating that it is more effective than the reference drugs. This same trend was observed in the reaction time of the studied plant.

Future work can be targeted on the analgesic property using other animal models which include anti-inflammatory, hot plate and tail flick methods.

In conclusion, the methanol leaf extract of *Clerodendrum volubile* has analgesic potential, thus validating its traditional use in pain management.

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