**http://dx.doi.org/10.4314/bajopas.v7i1.5**

***Bayero Journal of Pure and Applied Sciences, 7(1): 19 – 23***
*Received: February 2014*
*Accepted: June 2014*
**ISSN 2006 – 6996**

# PHYTOCHEMICAL SCREENING AND PRELIMINARY EVALUATION OF ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF THE METHANOL ROOT EXTRACT OF *CISSUS POLYANTHA*

**Sani, Y.M.[1], Musa, A. M.[1], Pateh, U.U[1],. Haruna,[1] A.K.,Yaro, A.H.[2], Sani, M.B[3]., Haruna, A.[3] and Magaji, M.G.[2]**

[1]Department of Pharmaceutical and Medicinal Chemistry, Ahmadu Bello University, Zaria-Nigeria.
Department of Pharmacology, Faculty of Medicine, Bayero University, Kano-Nigeria.
[2]Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria.
[3]Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria-Nigeria.
*Correspondence author: sanih20@gmail.com

***Cissus polyantha is used in traditional medicine for the treatment of conjunctivitis and inflammation. In this study, the methanolic root extract of Cissus polyantha was subjected to preliminary phytochemical screening, analgesic and anti-inflammatory studies. Phytochemical studies was carried out using standard phytochemical protocol while the analgesic studies was carried out using acetic acid-induced writhing tests in mice. Carrageenan-induced hind paw oedema in rats was used to evaluate the anti-inflammatory potential of the extract. Phytochemical studies of the methanolic crude root extract of the plant revealed the presence of carbohydrates, flavonoids, saponins, tannins steroids and triterpenes. The extract at doses of 20, 40 and 80 mg/kg, i.p significantly (P < 0.05) decreased the acetic-acid induced writhing. The extract also produced significant (P < 0.05) and dose-independent anti-inflammatory activity comparable to that of reference drug, ketoprofen. The intraperitoneal lethal dose (LD $_{50}$) toxicity studies on the methanol crude root extract of the plant was found to be 288.53 mg/kg body weight. These findings are suggestive of the analgesic and anti-inflammatory potentials of the methanol root bark extract of the plant and provide a scientific rationale for the use of the root of Cissus polyantha in traditional medicine.***

***Keywords: Cissus polyantha, Phytochemical screening Analgesic, Anti-inflammatory, traditional***

## INTRODUCTION

The plant kingdom having been explored for compounds of medicinal value for many years still undoubtedly has many species of plants containing substances of medicinal value that are yet to be discovered. Large numbers of plants are constantly being screened for phytochemical constituents and pharmacological activities (Trease and Evans, 1996). There is an increasing scientific interest in extraction and isolation of secondary metabolites from plants, as a part of biosynthetic, biochemical, pharmacological, chemotaxonomic, ecological, and phytochemical and plant tissue culture studies. As a result of modern isolation and pharmacological testing procedures, new plants usually find their way into medicine as purified substances rather than in the form of older galenical preparations (Silva *et al.,* 1998).

There is a continuous need to carry out research into these useful medicinal plants which are used in the therapeutic management of pains in order to obtain potent analgesics, with fewer side effects, cost effective and accessibility. Pain can be defined as an unpleasant sensation that can be either acute or chronic and that is a consequence of complex neurochemical processes in the peripheral and central nervous system (Richard andMary, 2006). Pain can also defined as a subjective, unpleasant, sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage (Dorland and Newman, 1988). Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively. These drugs have serious limitation due to their side effects such as gastrointestinal irritation, tolerance and dependency (Howland and Mycek, 2006). Medicinal plants have been found to be useful traditionally in the effective management of pain. *Stylosanthes fruticosa*, *Ficus glomerata, Bougainvilla spectabilis and Polyalthia longifolia* are examples of some these plants (Dalziel, 1958).

The validation of the folkloric claims of these medicinal plants will provide scientific basis for the development of their bioactive constituents. These could provide novel lead compounds or precursors in drug development; one of such plants with ethnomedicinal claims in pains and inflammatory conditions is *Cissus polyantha*.

*Cissus polyantha* is a semi-woody climber; of the closed-forest from Sierra Leone to Southern Nigeria. Many species in genus *Cissus* have been reported to possess wide range of uses to mankind ranging from medicinal, and feeds for livestock. Sap from macerated leaves of *C. polyantha* has been used in Liberia for treatment of conjunctivitis while the decoction of the underground root and the leaves is used in the management of pain and inflammatory conditions (Burkill, 1995).

This study, therefore, aims at evaluating the analgesic and anti-inflammatory potential of the methanoicl root extract of *Cissus polyantha* in laboratory animals.

## MATERIALS AND METHODS

### *Plant Material*

The plant material was collected from Turunku, Igabi Local Government Area of Kaduna State. Nigeria, in June 2010. The plant was identified by Mallam Musa Muhammad of the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University Zaria-Nigeria by comparing with existing specimen (Voucher Specimen number: 616). A specimen was subsequently deposited for future reference.

### *Extraction of plant material*

The root of the plant was washed with water, sliced and air dried under shade for 14 days; it was then crushed into coarse powder using a pestle and mortar. Powdered root (1000g) was exhaustively extracted by continuous maceration with 2L methanol for ten days. The solvent was then removed at reduced pressure to give 150 g of dark brown solid subsequently referred to as *Cissus polyantha* root extract.

### *Preliminary Phytochemical Screening*

The presence of carbohydrates, glycosides, anthraquinones, saponins, flavonoids, tannins, steroids/triterpenes and alkaloids was tested by the simple and standard qualitative methods described by Trease and Evans (1996).

### *Animals*

Swiss albino mice (19-23 g) and Wistar rats (180-200 g) of either sex were used for this study. The animals were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria-Nigeria, kept and maintained under laboratory conditions of temperature, humidity and light, and were allowed free access to food and water *ad labium*. All experimental protocols were in accordance with Ahmadu Bello University, Zaria Research policy; and ethic and regulations governing the care and use of experimental animals as contained in "Principles of laboratory animal care" (NIH Publication no. 85-23, revised 1985). Mice were used for the analgesic study while rats were used for the anti-inflammatory study.

### *Drugs administration*

Extract, normal saline and ketoprofen were administered via the intraperitoneal route. All administrations were at volumes equivalent to 1 ml/kg (for rats) and 10ml/kg (for mice).

### *Groupings*

Animals were randomly divided into five groups each consisting of six animals. The first group served as control and was treated with normal saline. The second, third and fourth groups were administered the extracts (20, 40 and 80 mg/kg) while the fifth group was administered with the standard.

### Pharmacological Screening

### Acute Toxicity Study

The method of Lorke (1983) was adopted in the estimation of the median lethal dose of the extract. The study was divided in to two (2) phases; in the first phase nine mice of either sex were divided into three groups of three mice each. Group I received 1000 mg/kg extract while group II and III received 100 mg/kg and 10 mg/kg extract respectively. The mice were observed for signs and symptoms of toxicity and mortality for 24 hours. In the second phase,4 mice were divided into 4 groups, each consisting one mouse and treated based on the result of the first phase. The first received extract at a dose of 140 mg/kg, while the second, third and fourth groups received the extract at doses of 225 mg/kg, 370 mg/kg and 600 mg/kg respectively. The mice were observed for 24 hours. The median lethal dose ($LD_{50}$) was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose i.e. the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded (Lorke, 1983).

### Acetic Acid-Induced Writhing in Mice

Thirty minutes post-treatment with normal saline, extracts or ketoprofen (10 mg/kg) , each mouse was injected with 10 ml/kg of aqueous solution of acetic acid (0.6% v/v), intraperitoneally. The number of writhes produced by each mouse was counted for ten minutes after five minutes latency period (Koster *et al.,* 1959).The percentage inhibition of writhing was calculated using the following formula:

$$\%\text{Inhibition} = \frac{\text{Mean number of writhing (control)} - \text{Mean number of writhing (test)}}{\text{Mean number of writhing (control)}} \times 100$$

### Carrageenan-Induced Paw Oedema in Rats

Thirty minutes post treatment with normal saline (1 ml/kg), extract or ketoprofen (10 mg/kg), 0.1 mL of sterile saline suspension of 1% w/v carrageenan was injected into the sub planter surface of the left hind paw of each rat. Paw diameter was measured using vernier caliper at time 0, 1, 2, 3 and 4 hours after the carrageenan administration (Winter *et al.,* 1962).

### *Statistical Analysis*

The data were expressed as mean ± SEM. The results were analysed using one way ANOVA followed by Dunnet t-test for multiple comparison. P values less than 0.05 ($P < 0.05$) were considered indicative of significance.

## RESULTS

*Preliminary phytochemical screening*

The preliminary phytochemical screening of the extract revealed the presence of flavonoids, tannins, saponins, steroids/triterpenes and cardiac glycosides.

**Acute toxicity Study**

The median lethal dose of the methanol root extract of *Cissus polyantha* was estimated to be 288.53 mg/kg. The animals showed respiratory depression before death.

**Acetic Acid-Induced Writhing Test**

The extract at the doses of 20, 40 and 80 mg/kg body weight significantly decreased the number of abdominal constrictions in mice. The extract reduced the mean number of writhes from 15.8 ± 1.77 in normal saline treated group to 3.4 ± 2.30, 2.4 ± 1.50, 1.0 ± 0.77 at doses of 80, 40 and 20 mg/kg body weight respectively. Highest reduction in abdominal constriction was observed at a dose of 20 mg/kg body weight which corresponds to 93.7% inhibition. Ketoprofen, the standard non-steroidal analgesic and anti-inflammatory drug also significantly decreased the number of abdominal constriction when compared with normal saline (Table 2).

**Carrageenan-Induced Paw Oedema in Rats**

The extract significantly reduced the increase in paw diameter induced by carrageenan at all the doses tested (20, 40 and 80 mg/kg body weight) when compared with normal saline treated group. Ketoprofen also significantly reduced the paw diameter of carrageenan induced inflammation in rats when compared with normal saline group. Both the extract doses and Ketoprofen significantly decreased the paw diameter of carrageenan induced inflammation in rats throughout the duration of the experiment when compared with normal saline group (Table 3).

**Table 1: Phytochemical Constituents of methanol extract of the root of *Cissus polyantha***

| TESTS | RESULTS |
|---|---|
| Carbohydrates | + |
| Glycosides | + |
| Saponins | + |
| Steroids and Triterpenes | + |
| Flavonoids | + |
| Tannins | + |
| Anthraquinones | – |
| Alkaloids | – |

KEY:+ Present,-absent

**Table 2: Effect of Methanol Crude Extract of *Cissus polyantha* Root on Acetic Acid-Induced Writhing in Mice.**

| Treatment | Dose (mg/kg) | Mean ± SEM | Percentage inhibition (%) |
|---|---|---|---|
| Normal saline | 10 | 15.8 ± 1.77 | - |
| Extract | 20 | 1.0 ± 0.77[a] | 93.7 |
| Extract | 40 | 2.4 ± 1.50 [a] | 84.8 |
| Extract | 80 | 3.4 ± 2.30 [b] | 78.5 |
| Ketoprofen | 10 | 4.8 ± 3.84 [c] | 69.6 |

Values were analyzed using student's t- test and presented as Mean ± SEM, a, b and c represent [a]P < 0.001, [b]P < 0.01 and [c]P < 0.05 respectively, n=5

**Table 3: Effect of Methanol Crude Extract of *Cissus polyantha* Root on Carrageenan- Induced Paw Oedema in Rats.**

| Treatment(mg/kg) | Mean paw Oedema (mm) | | | |
|---|---|---|---|---|
| | 1hr | 2hr | 3hr | 4hr |
| Normal saline10 (ml/kg) | 0.09 ± 0.007 | 0.29 ± 0.02 | 0.34 ± 0.02 | 0.28 ± 0.02 |
| Extract 80 | 0.07 ± 0.01[a] | 0.16 ± 0.03[d] | 0.11± 0.02[e] | 0.08 ± 0.01[d] |
| Extract 40 | 0.09 ± 0.01 | 0.26 ± 0.10[f] | 0.12 ± 0.007[a] | 0.09 ± 0.01[d] |
| Extract 20 | 0.06 ± 0.01 [b] | 0.13 ± 0.01[c] | 0.10 ±0.007[b] | 0.08 ± 0.008[b] |
| Ketoprofen10 | 0.05 ± 0.01 [c] | 0.08 ± 0.008[b] | 0.05 ±0.008[b] | 0.05 ± 0.008[b] |

Values were analyzed using student's t- test and presented as Mean ± SEM, a, b ,c, d, e and f represent P < 0.1, P < 0.01, P < 0.001, P < 0.02, P < 0.002 and P < 0.2 respectively, n=6

**DISCUSSION**

The data presented in this study showed that the crude root extract of *Cissus polyantha* possesses significant analgesic activity. The writhing responses of the mice to intraperitoneally injected noxious chemicals such as acetic acid can be used to screen for central and peripheral analgesic activity (Trongsakul *et al.,* 2003). The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesic (Gene *et al.,* 1998). In general, intraperitoneal administration of acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P, which stimulate nerve endings.

The abdominal constriction response is also thought to be mediated by local peritoneal mast cells, acid sensing ion channels and the prostaglandin (Bentley, *et al.,* 1983). It also causes increased levels of prostanoids ($PGE_2$ and $PGE_2\alpha$) in peritoneal fluids (Derardt, *et al*., 1980), as well as release of lipoxygenase products (Insel, 1996).

The extract may be said to behave like NSAIDs in these studies, thus, validate the ethnomedical use of the plant in painful inflammatory conditions (Hassan *et al.,* 2008). The most widely used primary test for screening of anti-inflammatory agents is carrageenan-induced rat paw oedema (Winter *et al.,* 1962). The development of oedema in the paw of the rat after carrageeenan injection is believed to be biphasic event. The initial phase observed during the first hour is attributed to the release of histamine and serotonin; the second phase is due to the release of prostaglandin-like substances (Antonio and Brito, 1998). Based on this, it could be argued that the suppression of first phase may be due to inhibition of cyclooxygenase (Ueno *et al*., (2002), found that the injection of carrageenan into rat paw induces the liberation of bradykinin, which later induces the biosynthesis of prostaglandins and other autocoids, which are responsible for the formation of inflammatory exudates. The extract produced significant inhibition of oedema in both phases of inflammation (1 hour and 3 hour). Therefore, it is suggested that the mechanism of action may be related to inhibition of histamine and serotonin release as well as prostaglandin synthesis inhibition.

The preliminary phytochemincal screening revealed the presence of anthraquinones, carbohydrates, flavonoids, glycosides, tannins, saponins and terpenes. Majority of the plants having flavonoids as their bioactive constituent(s) have been shown to possess analgesic and anti-inflammatory activities (Ahmadiani *et al,* 1998). Also flavonoids, saponins and tannins have been shown to exert analgesic effect on acetic acid induced writhing test (Calixto *et al*., 2000). The flavonoids, saponins and tannins might be responsible in part for the observed analgesic and anti-inflammatory effect.

Preliminary phytochemical screening indicated the presence of flavonoids in methanol crude extract of *Cissus polyantha* root. Selected phenolic compounds and flavonoids were shown to inhibit the cyclooxygenase and 5-lipoxygenase pathways. This inhibition reduces the release of arachidonic acid (Yoshimoto *et al*., 1983).

Eicosanoids such as prostaglandins are involved in various immunologic responses and are end product of cyclooxygenase and lipoxygenase pathways (Formica and Regelson, 1995). Flavonoids also inhibit both cytosolic and membranal tyrosine kinases which play key role in the signal transduction pathway that regulates cell proliferation (Formica and Regelson, 1995). Further, flavonoids are able to inhibit neutrophils degranulation and thereby decrease the release of arachidonic acid (Derardt *et al.,* 1980).Thus, the presence of flavonoids in the methanol crude extract of *Cissus polyantha* root might be responsible for the anti-inflammatory and analgesic activities observed.

The therapeutic benefits of traditional remedies are often attributed to combination of bioactive constituents (Chindo *et al.,* 2003) and might probably contribute in part to the analgesic and anti-inflammatory activities of the extract.

**CONCLUSION**

The methanol root extract of *Cissus polyantha* contains bioactive constituent(s) with relevant analgesic and anti-inflammatory activities as claimed from the folkloric investigation earlier reported and is worthy of further attention.

**REFERENCES**


Ahmadiani, A., Fereidoni, M., Semnanian, S., Kamalinejod, M. and Saremi, S. (1998). Anti-nociceptive and anti-inflammatory effects of *sambucus ebulus* rhizome extracts in rats. J Ethnopharmacol 61: 229-235.

Brito, A.R.M.S. and Antonio, M.A. (1988). Oral anti-inflammatory and anti-ulcerogenic activities of hydroalcoholic extract and partition fraction of *Turnera ulmifolia* (Turneaceae). *Journal of Ethnopharmacology*, 61: 215-228.

Bentley, G. A., Newton, S. H., and Starr, J. (1983).Studies on the anti-nociceptive action of agonist drugs and their interactions with opioid mechanisms.*British journal of pharmacology,* 32:295-310.

Burkill H.M. (1995). The Useful Plant of West Tropical Africa, Royal Botanical Gardens Kew, vol 3. Pp 94-98.

Calixto, J.B, Beirith, A. Ferreira, J. Santos, A.R. Cechinel, F.V. and Yuns R.A. (2000) Naturally occurring antinociceptive substances from plants Phytotherapy Research 14; 401-418.

Chindo, B.A., Amos, S., Odutola, A.A, Vongtau, H.O., Abbah, J., Wambebe. C. and Gamaniel, K.S. (2003), Central Nervous System activity of the methanolic extract of *Ficus platyphylla* stem bark *J. Ethnopharmacol,* 85:131-137.

Dalziel, J.M. (1957). The Useful Plant of West Tropical Africa. Crown agents for overseas government and administration. Mill Bank, London 2nd edition.

Derardt, R., Jongneg, S., Pelevalcee F. and Failhout, M. (1980). Release of prostaglandins E and F in an analgesic reaction and its inhibition. *Eur. J. Pharmacol.* V. 51: 17-24.

Dorland I. and Newman W.A. (1988). *Dorland's Illustrated Medical Dictionary.* 27th Edition. W.B. Sanders Company.Pp.54

Formica, J.V. and Regelson, W (1995). Review of the biology of Quercetin and related bioflavonoids. *Food Chem. Toxicol* 33(12):1061-1080.

Gene, R. M., Segura, L., Adzet, T., Marin, E. and Inglesias, J. (1998). *Heterotheca inuloides:*Anti-inflammatory and analgesic effects. *Journal of Ethnopharmacology,* 60**:** 157-162.

Hassan, A., Ahmad, I., Khan, A.M. and Choudhary, M.I. ( 1996). Two flavonol triglycoside from flowers of *Indigofera hebepetela. Phytochemistry,* 43(5): 1115-1118.

Insel, P.A. (1996). Analgesic-anti-inflammatory and anti-pyretic agents and drugs employed in the treatment of gout. In; Hardman, J.G.Limbird, L.E., Molinoff, P.B., Ruddon, R.W. and Gillman A.G. (Eds.). *Goodman and Gilmann The pharmacological basis of Therapeutics*.11th edition Mc Graw Hill Co Inc., New York (USA), Pp 617-657.

Howland, R.D. and M.J. Mycek, 2006. *Lippincott's Illustration Review: Pharmacology*. 3rdEdn., Lippincott Williams and Wilkins, London, ISBN-13: 9780781741187, pp 552.

Koster, R., Anderson, M. and De Boer, (1959). Acetic acid for analgesic screening. Federal proceedings. 18: 412-417.

Lorke, D. G. (1983). A new approach to practical acute toxicity testing. Archieve of Toxicology. 54. Pp275-287

Pharmacopoeia. First edition. Vol.1,pp XXXIX.

Silva, G.L., I. Lee and K.A. Douglas, (1998). Special problems with extraction of plants.In: Cannel, J.P.R. (ed.). *Natural Products Isolation.* Humana press publishers, New Jersey (USA). Pp 356-358.

Trease, G.E and Evans, W.C (1996), *Trease and Evans pharmacognosy* 14th edition w.b Ssuvnder company limited, London Pp191-293.

Trongsakul, S.A., Panthong, D., Kanjanapothi, T. and Taesotikul, T. (2003): The analgesic, antipyretic and anti inflammatory activity of *Diospyros variegate. J. Ethnopharmacology* 85 : 221-225.

Ueno, A.H; Naraba Y., Ikeda, F., Ushikubi, T. Murtala, S., Naramiya, S and Ohishi, S (2001). Intrinsic Prostacyclin contributes to exudation induced by bradykinin or Carrageenan: A study on the paw oedema-induced in ip-receptor-deficient mice. Life Sci., 66:155-160

Winter, E.A., Risley, E.A., Nuss, G.B. (1962). Anti-inflammatory and antipyretic activities of indomethacin. *Journal of Pharmacology and Experimental Therapeutics,* 141**:** 369-376

Yoshimoto, T.M.,Furukawa,S., Yamamoto,T and Watanabe-kohno, S. (1983). Flavonoids: Potent inhibition of archidonate lipoxygenase, *Biochem. Biophys. Res. Commun.*, 116:612-618