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Evaluation of the analgesic activity of ethanol extract of the stem and leaf of *Cissus gracillis* Gull and Perr (Vitaceae)

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Abstract

Cissus gracillis is used in the treatment of lumbago and pain. The analgesic activity of the ethanol extract of the stem and leaf of this plant was determined on mice and albino rats using radiant heat tail flick and acetic acid induced writhing assays. Extract was administered at doses of 125, 250 and 500 mg/kg through oral route. Phytochemical screening was done. Phytochemical screening showed presence of carbohydrates and terpenoids/steroids. In the tail flick assay, the difference in values was not statistically significant ($P < 0.05$, $P < 0.001$, $P < 0.01$) when compared with positive and negative control groups. There was a statistically significant ($P<0.05$) difference in the mean number of writhes between the extract treated groups and the negative control group. There was no statistically difference in the latency of the animals in the control and treatment groups in the tail flick assay. The findings of this study showed that ethanol extract of *C. gracillis* had significantly potent peripheral analgesic activity.

Keywords: *Cissus gracillis*; Tail flick; Acetic acid; Analgesic; Activity

1. Introduction

Plants have been increasingly useful in the treatment of various diseases and have served as essential components of pharmaceutical products as a result of varied biologically active constituents [1].

Traditional system of medicine continues to be widely practiced as a result of many reasons which include increase in population, low availability of orthodox drugs, low cost of treatment, several adverse effect of synthetic drugs and sometimes resistance to available drugs are seen with certain infectious diseases.

Some challenges associated with the use of medicinal plants include; lack of scientific proof of efficacy, lack of precise dosage, insufficient data, deforestation, and ease of adulteration.

Pain is a component of several chronic diseases and must be well treated so as to improve the quality of life and treatment outcome. Non-steroidal anti-inflammatory drugs and the opiates are usually employed in the treatment of pain [2]. These classes of drugs still present their unwanted adverse effects [3].

Several analgesic drugs are known today, but the desire to produce a better drug with lesser adverse effect initiated the interest on this plant. Shorinwa and Emenu, [4] reported the antidiabetic and antihyperlipidaemic activity of the stem and leaf of *C. gracillis*. There have been claims in the traditional medicine that the plant *Cissus gracillis* has analgesic

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property. *C. gracillis* is known as Okwukwo-iri nwere-omughor in Ikwerre, Rivers State, Nigeria [5]. The leaves of *C. gracillis* are occasionally eaten as vegetable in Sudan but its use as vegetable is limited elsewhere. The decoction of the leaves can be used for the relief of painful menstruation in women, its infusion can be used in the relief of child birth labor [6]. Hence, this study is aimed to scientifically investigate the analgesic activity of the leaves and stem of *Cissus gracillis*.

2. Material and methods

2.1. Plant material collection

The stems and leaves of the plant *C. gracillis* were collected from Obelle town in Emohua Local Government Area of Rivers State, Nigeria in their natural habitat. The plant was authenticated by Dr. Ekeke Chimezie of the Department of Plant Science and Biotechnology, University of Port-Harcourt with voucher specimen number (UPH/V/1249).

2.2. Extraction of plant material

The plant materials collected were cut into smaller pieces, air-dried under room temperature for two weeks and pulverized by grinding with the aid of a mechanical grinder. The powdered material (3.7 kg) was macerated with 17.84 L of absolute ethanol for 72 hours ensuring intermittent shaking of the macerating jars. The extract was concentrated using rotary evaporator. The concentrated extract was carefully evaporated to dryness over a water bath at 40˚C. The weight of the dried extract was measured, recorded and the extract was stored properly in airtight container which was then stored in the refrigerator.

2.3. Animals used

Albino rats of average weight (180g) and mice of average weight (22 g) of both sexes were obtained from the animal house of the Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port-Harcourt. All animals were housed in cages of five animals of same sex each at normal room temperature in the animal house of Department of Experimental Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of Port-Harcourt. It was ensured that the animals were fed with the right feed and water *ad libitum*. The animals were allowed to acclimatize before the commencement of the study.

2.4. Drugs used

- Acetyl salicylic acid 300 mg tablet (Emprin®) Emzor. Nigeria.
- Diclofenac sodium 100 mg tablet (Olfen-50®). Acino. Switzerland.

2.5. Phytochemical screening

Phytochemical screening was carried out on the ethanol extract of *Cissus gracillis* for the detection of various plant constituents [7].

2.6. Experimental protocol

Shorinwa and Emenu, [4], reported that the LD50 of ethanol extract of the stem and leaf of *C. gracillis* was greater than 5000 mg/kg. Hence the following doses of 250, 500 and 1000 mg/kg were selected based on the 1/5th, 1/10th and 1/20th of the LD_{50} of the plant extract.

2.7. Analgesic activity

2.7.1. Radiant heat tail flick method

Albino rats of both sexes were allocated into five different groups of five animals each. Ugo-basile tail flick analgesiometer was used to assess the tail flick latency of the rats. The route of administration adopted was oral. The negative control group was given 0.5 ml of distilled water while the positive group was administered with 10 mg/kg of diclofenac sodium. The test groups were treated with 125, 250 and 500 mg/kg of *C. gracillis* extract. The tail flick latency or reaction time was taken immediately for all the animals before administration of the extract, standard drug as well as the distilled water respectively. An infrared (IR) intensity of 50 and cut-off time of 20 seconds was fixed to prevent tissue injury during the process [8]. Radiant heat applied to the tail was maintained at 2 cm measured from the tip of the tail. and readings were taken at 30, 60, 90, 120, 150, 180, 210 and 240 minutes after administration. The time at which each animal withdrew its tail from the radiant heat source was taken as the reaction time or tail flick latency. The

analgesia produced by the test and standard drug was expressed by the difference in tail flick latency or mean increase in latency after drug administration [9].

2.7.2. Acetic acid induced writhing

Twenty- five mice of both sexes were assigned into five groups of five animals each. The test groups were administered with 125, 250 and 500 mg/kg of *C. gracillis* while the positive control group received 100 mg/kg of acetyl salicylic acid and distilled water was given to the negative control group. Oral route was employed in this study. One hour after the administration of each of these agents; 10 ml/kg of acetic acid in normal saline (0.6%v/v) was given intraperitoneally to each of the animals and number of writhes in thirty minutes was counted with intermittent recording of the value obtained every five minutes. A writhe is said to have occurred if there is a twisting movement of the abdomen or stretching of the hind-limb [10]. Percentage inhibition was calculated as follows:

$$Inhibition\ (\%) = \frac{Number\ of\ Writhes\ [Control] - Number\ of\ Writhes\ [Treatment]}{Number\ of\ Writhes\ [Control]}\ x\ 100$$

2.8. Statistical analysis

The results were expressed as mean ± SEM. Analysis of data was done using one-way analysis of data (ANOVA) followed by Dunnett's t-test. P-values were considered to be statistically significant at a value of P< 0.05.

3. Results

3.1. Phytochemical screening

Phytochemical screening of the extract revealed the presence of steroids/triterpenoids and carbohydrates while flavonoids, tannins, alkaloids, saponins and anthraquinones were not present.

3.2. Analgesic activity evaluation of *C. gracillis* by tail-flick method

There was no statistically significant difference in the mean latency between the extract treated groups and the control groups (Table 1).

Table 1 Analgesic activity (Mean ± S.E.M) of *Cissus gracillis* using tail flick method

	Dose	Reaction time in seconds (Mean ±SEM)							
Treatment	**(mg/kg)**	**0 min**	**30 mins**	**60 mins**	**90 mins**	**120 mins**	**150 mins**	**180 mins**	**240 mins**
Distilled water	0.5 ml	6.04 ± 0.77	8.62 ± 2.21	9.7 ± 2.16	6.42 ± 1.88	7.82 ± 3.00	7.26 ± 2.14	5.46 ± 1.32	4.85 ± 0.69
Diclofenac sodium	20	6.42 ± 1.31	10.58 ± 2.63	8.4 ± 1.54	7.3 ± 0.47	6.98 ± 1.19	6.12 ± 1.10	6.76 ± 2.21	4.96 ± 0.38
Extract	125	7.84 ± 2.1	12.74 ± 2.24	6.3 ± 0.82	5.62 ± 0.34	7.04 ± 1.36	6.52 ± 1.06	4.34 ± 0.30	5.18 ± 0.97
Extract	250	6.96 ± 0.57	9.36 ± 1.02	6.22 ± 0.81	5.94 ± 0.83	5.5 ± 0.46	5.2 ± 0.57	4.26 ± 0.55	7.02 ± 1.49
Extract	500	6.58 ± 1.29	9.2 ± 2.43	7.02 ± 0.85	7 ± 0.93	5.92 ± 0.86	9.04 ± 2.83	6.06 ± 0.92	7.04 ± 1.79

n =5, *P < 0.05, **P < 0.01, ***P < 0.001

3.3. Analgesic activity of *Cissus gracillis* on acetic acid induced writhing

There was a statistically significant (P<0.05) difference between the extract treated groups when compared to the negative control whereas the comparison done with the positive control group showed no statistically significant differences. The percentage inhibition of acetic acid induced abdominal constrictions produced by the extract increased with increase in dose (Table 2).

Table 2 Analgesic activity (Mean ± S.E.M) of the ethanol extract of *Cissus gracillis* by acetic acid induced writhing method

Treatment	Dose (mg/kg)	No of writhes (Mean ±SEM)	Reduction in writhes (%)
Distilled water	10ml/kg	229.4 ± 11.15	0
Acetylsalicylic acid	100	49.8 ±1.41*	78.29
Extract	125	79.8 ± 0.04*	65.21
Extract	250	61.6 ± 2.80*	73.1
Extract	500	36.6 ± 0.68*	84.04

n=5, *= P<0.05.

4. Discussion

The use of plants in the treatment of diseases is as old as mankind. Plants as the source of medicine in the world dates back to centuries [11]. Some phytochemicals serve as lead compounds for the synthesis of drugs useful in orthodox medicine [12]. Certain factors like availability, accessibility, and affordability have resulted in increased demand and usage of plants that have medicinal properties [13].

The preliminary phytochemical screening of the extract showed the presence of carbohydrates and steroids (triterpenoids).

An analgesic is a class of drug used in the reduction of pain and does not cause anesthesia. This class of drug has been proven to work on the central and peripheral nervous system of humans.

The radiant heat tail flick method is used to measure the potential of analgesic agents or substances by observing the reaction to heat [14]. It helps in ascertaining the effectiveness of a drug on pain threshold by measuring the degree to which the drug is able to increase the pain threshold [15].

The result obtained from this model could not explain clearly if this extract has central analgesic activity. This is because the comparison done between the diclofenac sodium treated group and extract treated groups showed no statistically significant difference. However, the greatest concern in this model is that there was also no significant difference between the two control groups; that is, between negative and positive control. This finding is contrary to the work done by Sani *et al*., [16], which reported that crude root extract of *Cissus polyantha* possesses significant analgesic activity as well as anti-inflammatory activity. Although this result showed no statistically significant difference among the groups, but a critical look at the individual group means values obtained 30 minutes after the drugs administration suggested that the extract might have mild neuropathic analgesic effect even though not statistically significant which is the target of tail flick study [17].

The acetic acid induced abdominal constriction test is often employed in the analgesic evaluation of substances for peripheral activity which has been reported to be mediated through local peritoneal receptors. Pain is induced through the action of cyclooxygenases on arachidonic acid leading to the production of prostaglandins and lipoxygenases [18]. Prostaglandins and lipoxygenases promote increase in capillary permeability which causes pain and inflammation [19].

Acetic acid induced pain may also involve the release of other nociceptive mediators such as bradykinin, serotonin and histamine [20].

The extract at all the examined doses (250, 500 and 1000 mg/kg) exhibited a significant inhibition of acetic acid induced abdominal constrictions when compared with the negative control group. There was an increased inhibition of abdominal constrictions with increase in the dose of the extract, therefore the peripheral analgesic activity could be said to be dose dependent.

This finding is comparable to that of Mohamad *et al*., [21], which stated that *Papaver libanoticum* extract possessed analgesic property as it was able to reduce acetic acid induced writhing in the animals**.** A statistical comparison was also done to know if there was a statistically significant difference ($P < 0.05$) amongst the extract treated groups and it was discovered that no significance difference occurred. Hence, it can be deduced that the peripheral analgesic activity of

the aerial parts of *Cissus gracillis* might be through the inhibition of endogenous pain mediators. NSAIDs are known to inhibit cyclooxygenase enzymes in peripheral tissues, hence interfering with the mechanism of transduction of primary afferent nociceptors [22]. The mechanism of peripheral analgesic activity of *C. gracillis* may likely be due to the inhibition of the synthesis or the effect of endogenous substances that stimulate pain nerve endings. The *C. gracillis* extract contains steroids triterpenes) thus, the observed analgesic activity might be attributed to the presence of steroids (triterpenes) as its phytoconstituents [23].

5. Conclusion

The findings of this study have shown that the ethanol extract of the stem and leaf of *Cissus gracillis* seemed to possess mild neuropathic analgesic effect and potent peripheral analgesic effect.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that no conflict of interest exists.

Statement of ethical approval

A written approval was obtained from the University of Port Harcourt research ethics committee according to international standard.

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