Journal of Plant Science and Medicinal Plants
www.resjournals.org/JPSMP

Vol. 1(1): 1-7, December, 2016

Antibacterial, Toxicological and Antidiarrheal Evaluation of Leaf Extracts of *Cissampelos owariensis*

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Abstract

The antibacterial, toxicological and antidiarrheal activities of the extracts of *Cissampelos owariensis* were investigated. The plant extracts were prepared by cold merceration method using water and ethanol and screened against *Escherichia coli, Salmonella* Typhi and Shigella *dysenteriae* using the agar well diffusion method. The LD_{50} of the extract was determined using locke's method. The antidiarrheal effect was evaluated against castor oil induced diarrhea in experimental rats. The result of the study revealed the presence of secondary metabolites such as tannins, resins, flavonoid, Saponins, steroids and terpenes and glycosides. The extracts showed activities against the test organisms with diameter of zones of inhibition ranging from 9mm to 16mm. Ethanol extract demonstrated stronger activity against the test organisms. Toxicity studies of the extracts revealed that the plant exhibited no significant toxicity as no signs of toxicity such as inactiveness, dizziness or mortality was observed even at the highest dose of 5000mg/kg body weight. The antidiarrheal assay shows that the test is dose dependent as there was significant reduction in the number of defaecation and wet faecal matter in comparison to control. The percentage inhibition of the ethanol extract at higher dose level of 400mg/ml/kg body weight and 200mg/ml/kg body weight was found to be 72.41% and 31.93% respectively. These results suggest that the plant may not be toxic to man and could be a potential source of novel antidiarrheal agents. Based on the result of this research work, it is therefore recommended that the synergistic effect of the plant extract in combination with antibiotics and non-antibiotic-antidiarrheal agents respectively be evaluated.

Keywords: *Cissampelos owariensis*, Antibacterial, Antidiarrheal, Toxicity, Extracts.

INTRODUCTION

Herbal medicines are becoming very popular in the developing countries because of the shift from synthetic drugs to natural remedies. This is probably due to the perceived beneficial and lower side-effect profile of natural products that are extracted from plants (Ekpe *et al*., 1990). An herbal medicine is one that contains materials as a finished product and may include whole plant parts or other plant materials (Agada *et al*., 2005). Information on the use of medicinal plants has been obtained from herbalists, herb sellers and indigenous people of Africa over many years (Sofowora, 2000). The searches for medicinal plants that are more potent and efficient antibiotic agents have accelerated in recent

years. In Nigeria most medicinal plants are traditionally used in folk medicine to treat various diseases, (Alho and Leinonen, 1999).

Cissampelos owariensis is a plant under the family *Menispermaceae* which consists of about 70 genera and 450 species found in tropical regions. *C. owariensis* is found in the wild in some West African countries, including Ivory Coast, Zambia, Uganda, Mozambigue, Angola and Nigeria. In Nigeria, it is mostly found in parts of South-Western and North-Eastern Nigeria and has been used extensively in African traditional medicine. It occurs in low land and riverine forest, also in secondary forest, and it is often also common in clearings, orchards, fields and hedges, especially in moist soils up to 900 m altitude (Erhirhie *et al*., 2015; Akande *et al*., 2013).

It is commonly called velvet leaf in English. In Ghana, it is called *Akan-Asante* akuraso meaning mouse's ear; referring to the shape of the leaf. Other vernacular names include; "Damal gwaraajii" in Hausa and "Ewe jokoje" in Yoruba. *Cissampelos owarriensis* roots, bark and leaves are used as *anthelmentic*, *dysmenorrhoea,* sedative, gastrointestinal complaints such as diarrhoea, dysentery, colic, intestinal worms and digestive complaints and also urogenital such as menstrual problems, venereal diseases, infertility, to induce contraction of the uterus to start labour or abortion and also to expel the placenta (Habila *et al*., 2011).

In Nigeria leaf sap is used as nose or eye drops to cure headache. In Congo a decoction of stems mixed with the leaves of other plants is used as a wash to treat wounds. In south-eastern Tanzania grated rhizome is applied to snake bites. The aerial parts are used to treat amnesia and psychoses and in the preparation of health tonics. A decoction of crushed leaves is used in veterinary medicine to treat diarrhoea. In Nigeria the rhizome is sometimes used in the preparation of arrow poison (Akoègninou *et al*., 2006).

Antimicrobial drug resistance is not only on the increase but also a serious problem to the medical profession as infectious diseases such as diarrheal are the number one cause of death, accounting for approximately half of all death in tropical country (UNICEF/WHO, 2009). This situation has necessitated the search for antimicrobial agents from plant –based products (Okoro *et al*., 2012). Also, the problem of intoxication and inefficiency cases has also been on the increase on the usage of the medicinal plants. This may be due to overdosing or under dosing of the medicinal plants used. Therefore it is important to evaluate the activity of such plants against the pathogens to ascertain their activity (Gathu, 2006). Nature has provided many things for mankind over the years, including the tools for the first attempts at therapeutic intervention. Ancient civilization depended on Plant extracts for the treatment of various ailments. Today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or used as templates for the development of new therapeutic agents (Kumarasamy *et al.,* 2002). In Plateau State, infusion of *C. owariensis* is used in the treatment of diarrhea by some ethnic groups such as Birom, Afizere and Fulanis. It is against this backdrop that this study intends to assess the antibacterial, toxicological and antidiarrheal activities of *Cissampelos owariensis.*

MATERIALS AND METHODS

Collection and Identification of Plant

Careful selection of fresh leaves of *Cissampelos owariensis* free of blotting and infectious agents were obtained at Sheri hill in the month of August, 2015. The plant was authenticated by a plant Taxonomist at the herbarium unit of the Department of Federal College of Forestry, Bauchi road, Jos, Nigeria, where voucher specimen was deposited.

Collection, Identification and Preparation of Test Organisms

Clinical isolates of *Escherichia coli*, *Shigella dysenteriae* and *Salmonella* Typhi were obtained from stock cultures in the bacteriological unit of Federal College of Veterinary and Medical Laboratory Technology, Vom, Jos South Local Government Plateau State, Nigeria. The test organisms were confirmed using standard microbiological procedures (Chessbrough, 2006). A twenty four hours (24hr) culture of the bacterial culture isolate were diluted with physiological saline solution and the turbidity corrected by adding sterile physiological saline until a McFarland turbidity standard of 0.5 (10^6 CFU/ ml) were obtained (Cheesbrough, 2006).

Culture Media, Antibiotics and Solvents

Different types of media were required in the study, it include, Peptone water broth, Muller Hinton broth, Nutrient agar and Mueller-Hinton agar. Ethanol was used for extraction processes. These media and solvent were purchased from Medicom Laboratory Nigeria limited, Jos, Plateau State, Nigeria.

Preparation of Plant Extracts

The plant materials were air dried in the open (away from the sun) at room temperature for four weeks until a constant weight was obtained. These dried plants were pounded into fine powder using a mortar and pestle. They were then packaged in air tight containers, labelled accordingly and kept until when ready to be used.

Plant Extractions

Ethanolic extraction

Plant extracts were obtained using cold maceration method with intermittent shaking. Two hundred grams (200g) of the powdered materials was weighed using an analytical weighing machine and soaked in 1000ml of ethanol at room temperature for 24 hours then filtered with Whatman N0 1 filter paper pore size 11µm. The filtrate was evaporated in a rotatory flask evaporator and dried in a desiccator which yielded dry weight residue of plant extract. The dried extract was preserved in a refrigerator at a temperature 35°C till required for antimicrobial screening.

Aqueous extraction

Plant extracts were obtained using cold maceration method with intermittent shaking. Two hundred grams (200g) of the powdered materials was weighed using an analytical weighing machine and soaked in 1000ml of distilled water at room temperature for 24 hours then filtered using Whatman N0 1 filter paper pore size 11µm. The filtrate was evaporated in a rotatory flask evaporator and dried in a desiccator which yielded dry weight residue of plant extract. The dried extract was preserved in a refrigerator at a temperature 35°C till required for antimicrobial screening.

Determination of Phytochemicals

Qualitative phytochemical analysis of the plant extracts of the plant was determined by the methods used by Edeoga *et al.,* (2005); Jigna and Sumitra, (2007).

Preparation of Plant extracts Concentrations

One gram (1g) of each aqueous and ethanol extracts pre-prepared (each separately) was taken and the aqueous extract was dissolved in 10ml sterile distilled water, while the ethanol extracts were dissolved in 20% DiMethyl Sulphoxide (DMSO). Thus 100 mg / ml of stock was obtained as a standard concentration of aqueous and ethanol extracts respectively. Different working concentrations (50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml) were prepared using doubling dilution of the prepared stock solution of 100mg/ml concentration.

Antimicrobial Activity Assay of Plant Extract

The agar-well diffusion of Bauer (1996) was adopted. Holes of 6 mm in diameter were made in the seeded agar using a 6mm cork borer. The base portion of the hole was then sealed with molten nutrient agar to ensure adequate diffusion. Aliquot of 20 µl from each concentration of extracts were added into each well using a micropipette on the seeded medium and allowed to stand on the bench for 1 h for proper diffusion and thereafter incubated at 37^0C for 24 h. They were observed for zones of inhibition which were measured in millimeters (mm) using a transparent ruler and values were tabulated.

Acute Toxicity Studies

The LD_{50} of the ethanol extract of *Cissampelos owariensis* was determined using the method of Lorke (1983) in consonance with OECD guideline on animal acute toxicity testing (OECD, 2001). Twelve (12) Wistar albino rats weighing between 150-200g were used. In the first phase, animals were fasted for 2hrs before the study, but were given

Table 1. Phytochemical constituents of the extracts of *Cissampelos owariensis*

Secondary metabolites	Ethanol Extract	Aqueous Extract
Flavonoids	+	+
Saponins	+	+
Carbohydrates	+	+
Tannins	+	+
Alkaloids	-	-
Cardiac glycosides	+	+
Steroids and terpenes	+	-
Resins	+	+
Antraquinones	+	-

Key:
(+) = Present in small amount
(-) = Absent

water *ad libitum*. Rats were divided into four groups of three rats each and were treated with the ethanol extract at different doses of 10, 100 and 1000 mg/kg (body weight) orally while the last group was then used as control. They were observed for 24hrs for signs of toxicity such as inaction, dizziness, loss of weight and mortality. In the second phase, 12 rats were divided into four groups of three rats each and were treated with the extract doses of 1500, 2900, and 5000 mg/kg (body weight). The LD_{50} was calculated using the formula below to determine any toxicity or mortality.

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where D_0 = Highest dose that gave no mortality,
D_{100} = Lowest those that produce mortality

Anti-diarrheal Test

Castor oil induced Diarrhea

The antidiarrheal activity of the extract was studied using the method of Awouters *et al* (1978). The *Wistar albino* rats weighing 150-200g of either sex were divided into four groups of five rats each and were treated as per the following regimen. Groups 1 and 2 were given graded doses of the extract (200mg/kg, 400mg/kg, respectively). Group 3 were given 2ml/100g distilled water – Negative control. Group 4, were given Loperamide (10mg/kg body weight orally positive control). The rats were housed singly in cages lined with white blotting paper. One hour after the above treatment, all the rats in the groups were then given castor oil orally. The rats were observed for 6 hours for watery (wet) or unformed faeces. The watery faeces from each rat were counted hourly for up to 6 hours. At the end of the experiment the group mean was obtained and the percentage of protection were calculated.

RESULTS

Phytochemical Screening

Table 1 shows the phytochemical results of *Cissampelos owariensis.* Preliminary phytochemical investigation of aqueous and ethanol extracts of the plant were compared. The result shows that resins were found to be the most abundant as they were found in higher amounts in all the extracts. Tannins, glycosides and flavonoids were present in higher amount in the aqueous extracts and in moderate amount in the ethanol extract. Saponins were also present in small amount for both extracts. Steroids and terpenes were present in moderate amount in the ethanolic extract but were absent in the aqueous extract. Similarly, Amtraquinones were present only in the ethanolic extract but absent in the aqueous extract. Alkaloids were however absent in the aqueous and ethanolic extracts.

Table 2. Antibacterial activities of *Cissampelos owariensis* against *S. dysentriae, E. coli* and *S. Typhi.*

Test organisms	Aqueous Extract					Ethanoic Extract					Control	
Conc.(mg/ml)	100	50	25	12.5	6.25	100	50	25	12.5	6.25	+ve	-ve
E. coli	12	10	10	9	8	14	12	9	8	8	21.5±2.12	0.0
S.dysenteriae	16	13	11	9	9	18	16	15	13	12	25.00±5.65	0.0
S. Typhi	12	9	8	0.0	0.0	16	14	13	10	9	26.00±2.82	0.0

Key: +ve control = Ciprofloxacin (0.625mg/ml), -ve control = DMSO, Conc = concentration

Table 3. Acute toxicity test of extract of *Cissampelos owariensis*

PHASE 1		
Doses (mg/kg)	Result	Remark
10	No signs of toxicity or mortality observed	3/3
100	No signs of toxicity or mortality observed	3/3
1000	No signs of toxicity or mortality observed	3/3
PHASE 2		
1500	No signs of toxicity or mortality observed	3/3
2500	No signs of toxicity or mortality observed	3/3
5000	No signs of toxicity or mortality observed	3/3

Key:
3/3 = All survived

Antibacterial Assay of Plant Extracts

Table 2 depicts the antibacterial activity of the extracts of *C. owariensis* at varying concentrations against *E. coli*, *S. dysenteriae* and *S*. Typhi. The result generally shows that both aqueous and ethanol extract demonstrated a good antibacterial properties although the ethanol extract shows higher activity than the aqueous extract. The ethanol extract showed activity with zone of inhibition ranging from 8mm-14mm against *E. coli,* 12mm-18mm against *Shigella dysenteriae* and 9mm – 16mm against S. Typhi at the concentrations used respectively. The zone of inhibition ranges from 8mm-12mm and 9-16mm for aqueous extracts against *E. coli* and *Shigella dysenteriae* respectively. No antibacterial activity was observed at concentrations of 12.5mg/ml and 6.25mg/ml against *S*. Typhi as for the aqueous extract. The aqueous extract was however active against *S*. Typhi at a concentration of 100mg/ml, 50mg/ml and 25mg/ml as indicated by 12mm, 9mm and 8mm zones of inhibition respectively. The result also show that the susceptibility of the test organisms to the control drug (Ciprofloxacin, 0.625mg/ml) was higher than their susceptibility to the extracts at different concentrations.

Acute toxicity Test result

It was found that the ethanol extract of *C. owariensis* was not toxic because even at a very high dose of 5000mg/kg body weight, neither death nor other signs of toxicity were observed (Table 3).

Antidiarrheal Activity of *Cissampelos owariensis* extract

It was also observed that the ethanol extract at both dose levels of 400mg/ml and 200mg/ml significantly reduced number of defaecation and wet faecal matter in comparison to control. All the tested extracts showed dose dependent

Table 4. Antidiarrheal effect of the ethanolic extracts of *C. owariensis* on castor oil induced diarrhoea in rat.

Group	Drug and Dose	Number of faeces in 6 hrs	Number of wet faeces in 6 hrs	Percentage inhibition of wet faeces
I	200mg/kg	6.50 ± 0.223[b]	5.33 ± 0.21[a]	31.93
II	400mg/kg	3.83 ± 0.307[b]	2.16 ± 0.401[b]	72.41
III	2ml/100g D/W (-ve control)	10.83 ± 0.307	7.83 ± 0.401	0
IV	Loperamide (+vecontrol 10mg/kg)	1.66 ± 0.210[b]	1.01± 0.258[b]	87.23

Values are expressed as Mean ± SEM, n = 5 each group
[b]P < 0.01, [a]P<0.05 compared to vehicle control.
Significant association exists at P<0.05 or P < 0.01

effect. The percentage inhibition of the ethanol extract at higher dose level of 400 mg/ml/kg body weight and 200mg/ml/kg body weight was found to be 72.41% and 31.93% respectively. Results were compared with that of standard drug loperamide (Table 4).

DISCUSSION

The phytochemical analysis of *C. owariensis* revealed the presence of secondary metabolites such as tannins resins, flavonoid, Saponins, steroids and terpenes, glycosides which have been previously reported for their antimicrobial activities. This is similar to what was reported by Akande *et al*. (2013) who asserted that many plants have been reported for therapeutic purposes because of the chemical compounds synthesized in these plants. Hence, the observed antimicrobial activity of the plant extracts against the test organisms may be due to the presence of its phytochemical components. This study has shown that *Cissampelos owariesis* is used in the treatment of many infectious diseases and in particular diarrhea diseases. These plants demonstrates varying activities in terms of their inhibitory effects on *E. coli*, *Salmonella* Typhi and *Shigella dysenteriae*. The extract that showed stronger activity was the ethanol extract with zones of inhibition greater than 8mm against the test organisms.

Akande *et al*. (2013) had reported the antibacterial activity of crude extracts of *C. owariensis* root on *Staphyloccoccus aureus*, *Streptococcus pyogenes*, *Escherichia coli*, *Salmonella Typhi* and *Pseudomonas aeruginosa*. They attributed the inhibitory activity of the crude extract of the plant to the presence of tannins, flavoniods, Alkaloids, Saponins and carbohydrates. In a similar work by Habila *et al*. (2011), *Cissampelos owariensis* extract was shown to have antibacterial activity against clinical isolates; *Staphylococcus aureus, Streptococcus pyogene, Salmonella Typhi, Escherichia coli, Shigellia dysenteriae, Proteus vulgaris* and *Candida albicans*. They also reported bioactive components of the extracts as; alkaloids, saponins, triterpenes, flavonoids and glycosides.

The aqueous extract of *Cissampelos owariensis* has also been reported to have broad activity against gram positive and gram negative bacteria. The extract was shown to have activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Vibrio cholerae* and *Salmonella* Typhi. In their report, *Salmonella* Typhi was the least susceptible while *Staphyloccoccus aureus* was the most susceptible (Effiom *et al*. 2009).

Erhirhie (2015) also reported that an infusion of the bitter rhizome, leaves or stems of *Cissampelos owariensis* is used to cure gastrointestinal complaints such as diarrhoea, dysentery, colic, and intestinal worms. The fact that the ethanol extract had better inhibitory effect than the aqeuous simply shows that ethanol is the best solvent for the active components that has anti-diarrhea activity.

The study has also revealed that the plant is relatively non-toxic as no death or other signs of toxicity were observed in both stages of the experiment. It can thus be said that the LD_{50} of the extract is higher than 5000mg/kg body weight hence the extract is relatively non-toxic. According to locke, (1983), compounds of slight toxicity will have LD_{50} between 5000 and 15000mg/kg.

In the present investigation, anti-diarrhoeal activity was evaluated by castor oil induced diarrhoeal model. It was observed that the ethanol extract at both dose levels significantly reduced number of defaecation and wet faecal matter in comparison to control. The tested extracts showed dose dependent effect. The result of the study also shows that activity is dose dependent. Earlier studies reported that the root and leaf of the plant exhibited significant antibacterial activity (Akande *et al*., 2013; Habila *et al*., 2011; Erhirhie, 2015). This may also be responsible for the antidiarrhoeal activity.

CONCLUSION

The phytochemical results indicate the presence of metabolites such as tannins, resins flavonoids, saponins, steroids and terpenes and cardiac glycosides. The presence of these bioactive components in all the extracts tested could probably be responsible for the observed antibacterial and antidiarrheal activities. The ethanol extract showed higher activity than the aqeuous extract against the tested organisms. The plants exhibit no significant toxicity and demonstrated good anti-diarrheal agent. This plant therefore present a potential novel and cheap source of potent antimicrobial agents which could justify it been claimed for ethno medicinal uses.

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