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EVALUATION OF PHYTOCHEMICALS AND ANTI-INFLAMMATORY EFFECTS ON METHANOL *EXTRACTS* OF *Aeschynomene uniflora*

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ABSTRACTS
Studies on Aeschynomene uniflora was carried out with the aim to establish scientific validation by evaluating the phytochemical constituents, toxicity and anti-inflammatory effects of the Methanol extract in order to probe its ethnomedical uses. Various phytochemical constituents from the plant were evaluated using standard method which reveals the presence of carbohydrates, cardiac glycoside, flavonoids, saponins, steroids, triterpenes and tannins were present in the crude extract. Toxicity studies by using oral route showed no death in any group even at 5000 mg/ kg indicating its safety. Anti-inflammatory effects indicates its efficacy in which the dose at 1000 mg/kg showed more activity compared to Control followed by 250 mg/kg and then 500 mg/kg. The results obtained in this present study indicates the plant to have the potential to act as a source of useful drugs because of the presence of various primary and secondary metabolites .The results were very much encouraging but more scientific validation is necessary before being put into practice.
Key words: Aeschynomene uniflora, Anti-inflammatory, Phytochemicals, Toxicity

INTRODUCTION

Plants have an almost endless variety of metabolites which is very useful to human beings (Suresh and Sagadevan, 2011). The importance of plants is well known to us. Plant kingdom is a treasure house of potential drugs and in the recent years there has been an increasing awareness about the importance of medicinal plants. Drugs from the plants are easily available, less expensive, safe and efficient that rarely have side effects (Thite *et al*., 2013). Medicinal plants are rich source of bioactive phytochemicals or bio nutrients. Studies carried out during the past 2– 3 decades have shown that these phytochemicals have an important role in preventing chronic diseases like cancer, diabetes and coronary heart disease (Daniel and Krishnakumari, 2015). There is, however, much scope for further systematic research in screening Indian medicinal plants for these phytochemicals and assessing their potential in protecting against different types of diseases (Daniel and Krishnakumari, 2015). Medicinal plants are used by 80% of the world population for their basic health needs. *Aeschynomene uniflora* belongs to the family Fabaceae. It is an erect or ascending, rarely almost prostate, annual or short-lived plant found in several places in Africa, especially in fresh water swamp and aquatic vegetation (Burkill, 1985). The plant is used in traditional medicine for treatment of psychotic disorder, tuberculosis, skin infection, antidote to snake venom, menstrual disorder and small pox. The aqueous extract of the whole plant is administered topically over the whole body to cure small pox in northern Nigeria. The plant is eaten as vegetable to cure fever symptoms and cough in Benue state Nigeria.

Inflammation is a biological complex of vascular tissues by harmful or stimuli of pathogens and irritants (Meena *et al*., 2009) and has been major health problems in the world (Li *et al*., 2003). Although, several agents are known to treat inflammatory disorders, their prolonged use often leads to gastric intolerance, bone marrow depression, water and salt retention (Rajasekaran, *et al*., 2001).

Now a days herbal treatments are becoming increasing by popular as the herbal preparations have no or least side effects (Sharma *et al*., 2009). World Health Organization (WHO) estimates that 80% of the population relies on plant based products for human health care (Gurib-Fakim *et al*., 2006).

Aim

Is to establish the scientific validation for the use of *A. uniflora* in traditional medicine

Objectives

I) To evaluate the phytochemical constituents
II) To establish the safety or otherwise of methanol extract using Lorke's method
III) To establish the anti-inflammatory effects of the extract

MATERIALS AND METHODS

Collection and Identification of Plant material

Plant specimen collection were carried out in May, 2016 from Ringim Local Government Area of Jigawa State and were conveyed for identification, authentication at the Herbarium unit of Bio- resources, National Research Institute for Chemical Technology (NARICT), Zaria by a taxonomist in which a voucher's numbers 4561 were assigned to the plant.

Extraction of Plant Materials

Dried plant materials (1 kg) were extracted using cold maceration with 2.5 L Methanol. The contents were then be filtered with a filter paper (Whatman no.1), the filtrate was concentrated to dryness using water bath which was kept in desiccator.

Preliminary Phytochemical Screening

The various plant parts used traditionally were screened basically to detect the presence or absence of plant chemical constituents such as alkaloids, tannins, saponins, anthraquinone, flavonoids, steroids, terpenoids, carbohydrates and glycosides. The plant species selected for screening were based on the observed published literatures on chemical constituent's evaluation as either little or absent.

This procedure were carried out on the Methanol extract according to (Abimbola *et al*., 2013; Evans, 2009) as outlined below.

Alkaloids

Dragendorff's test; to 2 mg of the hexane extract 5 ml of distilled water were added, 2 ml of Hydrochloric acid were added until an acid reaction occurs. To this 1 ml of Dragendorff's reagent were added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

Wagner's test; 2 mg of hexane extract were acidified with 1.5 % v/v of hydrochloric acid and a few drops of Wagner's reagent were added. A yellow or brown ppt. indicates the presence of alkaloids.

Mayer's test; to a few drops of the Mayer's reagent, 2 mg of hexane extract were added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.

Flavonoids

Shinoda's test; 2 mg of hexane extract were dissolved in 5ml of ethanol and to this 10 drops of dilute hydrochloric acid followed by a small piece of magnesium were added. Formation of pink, reddish or brown colour indicates the presence of flavonoids.

Triterpenoids

Liebermann - Burchard's test; 2 mg of dry extract were dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid were added along the sides of the test tube. Formation of a pink colour indicates the presence of triterpenoids.

Saponins

In a test tube containing about 5 ml of an hexane extract, a drop of sodium bicarbonate solution was added. The test tube were shaken vigorously and left for 3 minutes. Formation of honey comb like froth indicates the presence of saponins.

Steroids

Liebermann-Burchard's test; 2 mg of dry extract was dissolved in acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid were added along the sides of the test tube. Formation of green colour indicates the presence of steroids.

Salkowski reaction; 2 mg of dry extract was shaken with chloroform, to the chloroform layer sulphuric acid were added slowly by the sides of test tube. Formation of red colour indicated the presence of steroids.

Tannins

To 1-2 mg of the hexane extract, few drops of 5% w/v $FeCl_3$ solution was added. A green colour indicated the presence of gallotannins, while brown colour indicates the presence of pseudotannins.

Toxicity Studies of the Plants Extract

The acute toxicity study were carried out using mice of both sexes and according to Lorke (1983) as outlined below.

- The stock solution of 1 mg of the hexane extract was dissolved in 10 ml of distilled water will be prepared with few drops of Tone 80 added to enhance the extract solubility.
- Serial dilution of 100 mg/ml and 1000 mg/ml of the extract were then be prepared.
- In the first phase, the mice were weighed and their weight was recorded. The animals was grouped in to 3 by 3 groups and the dose to be administered for each group of mice were then be calculated using the formula; Dose × Weight of mice in kg/ stock solution.
- The extract was administered to the animals according to the volume of dose to be administered as calculated above orally which was observed for any physical changes before allowed to stand for 24 hours and the number of death was recorded for each group.
- In the second phase, serial dilution of 200 mg/ml, 400 mg/ml, 800mg/ml and 1000mg/ml was prepared and administered to 1 mouse each per group. The administration of the extract were carried out as described above.
- The acute toxicity was then calculated using the formular below.

$$\sqrt{\text{minimumdose of death} \times \text{minimum dose of sur}}$$

Determination of Anti-inflammatory Activity using Carrageenan and Dextran Induced paw edema in mice

Freshly prepared 1% carrageenan or textran in 0.1 % carboxy methyl cellulose (0.02 ml) was injected on subplantar region of the right paw of mice to induce acute inflammation (Winter *et al.*, 1962 and Maity *et al.*, 1998) with some modifications. The animals were divided in to 8 groups, 6 animals each. Group I remained as control for carrageenan. Group II and III received 250 and 500 mg/kg body weight extract (0.1 ml in distilled water) orally by intubation guage 1 hr before carrageenan injection and group IV, positive control received 10 mg/kg body weight diclofenac (ip). Group V were remained as control for dextran model. Group VI and VII received 250 and 500 mg/kg body weight of the plants extracts (0.1 ml) orally by intubation gauge and group VIII (positive control) received 10 mg/kg body weight diclofenac (ip) 1 hr before dextran injection. The thickness of the paw were measured using a vaernier caliper before and after carrageenan or dextran injection and thereafter at every hour up to 6 hrs. But in this case we have taken 1000, 500 and 250 mg/kg to see how effective was the extracts at the lower, middle and the high doses and also the animals were divided in to five groups, 5 animals each, hence our extracts toxicity studies were practically non-toxic because even at 5000 mg/kg were no death encountered. Percentage of inhibition were calculated using formula below;

%inhibition of paw thickness = $[((tCn - tC0) - (tTn - tT0))/((tCn - tC0)$] x100.

STATISTICAL ANALYSIS

All data was expressed as mean ± SEM and one way Analysis of Variance Anova statistical test using SPPS to test the significance. $P<0.05$ was considered significance.

RESULTS AND DISCUSSION

Plate 1; Typical *Aeschynomene uniflora* **in its natural Habitat**

Table 1: Showing some phytochemical constituents from the Methanol extract

Test Constituents	Methanol
Carbohydrate	+
Cardiac glycoside	+
Tannins	+
Saponinns	+
Flavonids	+
Anthraquinone	+
Steroid	-
Triterpenes	-
Alkaloid	+

+ = present, - = absent

Acute Toxicity Study

The acute toxicity studies of the methanol extract gave no LD_{50} even at 5000 mg/kg in mice, using oral route of administration. The result was presented in the table below;

Table 2: Acute Toxicity Study of Methanol Extract of *Aeschynomene uniflora*

First Phase		Second Phase	
Dose (mg/kg)	**Mortality**	**Dose (mg/kg)**	**Mortality**
10	0/3	1600	0/1
100	0/3	2900	0/1
1000	0/3	5000	0/1

Table 3: Showing the anti-inflammatory effects of the Methanol Extract (Mean Paw diameter (mm) ±SEM at a different particular time intervals)

Treatment	Dose (mg/kg)	At 30 minutes	At 1 hours	At 2 hours	At 3 hours	At 4 hours	At 5 hours
Control	Carrageenan in D. Water	0.43± 0.040	0.488± 0.034	0.638± 0.061	0.758± 0.039	0.752± 0.039	0.648± 0.069
Aeschynomene uniflora	1000 mg/kg	0.148± 0.043***	0.248± 0.045**	0.392± 0.054**	0.51± 0.044***	0.302± 0.034***	0.272± 0.047***
Methanol Extract	500 mg/kg	0.17± 0.034***	0.274± 0.029**	0.396± 0.057**	0.55± 0.04**	0.46± 0.073***	0.412± 0.027**
	250 mg/kg	0.2± 0.027***	0.29± 0.049**	0.436± 0.037*	0.55± 0.038**	0.368± 0.052***	0.332± 0.039***
Diclofenac	10 mg/kg	0.229± 0.44	0.338± 0.049	0.434± 0.039*	0.422± 0.032***	0.386± 0.04***	0.27± 0.034***

n= 5, *** Signifies highly significance at P<0.05, **Signifies significance at P<0.05, D= Distilled water

The presence of the secondary metabolites in the crude extracts of this plant may be responsible for some of the biological activities observed (Musa *et al*., 2005). Phenolic compounds such as flavonoids and tannins which are presents in this plant are one of the largest and most ubiquitous groups of plant metabolites (Adejumomi *et al*., 2008). They possess biological properties such as antiapoptosis, antiaging, anticarcinogen, antiinflammation, antiatherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities (Han *et al*., 2007). This could explain the vast usage of this plant to manage infectious disease in folklore medicine.

Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Krings and Berger, 2001). Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc. This therefore implies that this plant could possess antiaging, anticarcinogenic properties (Ali *et al*., 2008). Saponins are known to produce inhibitory effect on inflammation and as such, the presence of saponins in the crude extracts of this plant shows that this plant could be used as an anti-inflammatory agent (Just *et al*., 1998). The presence of saponins in the crude extracts may be responsible for the significant anti-bacteria activity exhibited, as these bacteria are responsible for inflammations. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*., 2000 and Okwu, 2004). Steroids have been reported to have antibacterial properties (Epand *et al*., 2007), and they are very important compounds especially due to their relationship with compounds such as sex hormones (Okwu, 2001). Glycosides are known to lower the blood pressure according to many reports (Del-Rio *et al*., 1997). The results obtained in this study suggest that, the identified phytochemical compounds may be the bioactive constituents and this plant is proving to be an increasingly valuable reservoir of bioactive compounds of substantial medicinal merit (Marjorie, 1999). Plants have the ability to produce a large variety of secondary metabolites such as saponins, tannins, phenols, alkaloids, triterpens and phytosterols. For example, alkaloids protect against chronic diseases. Adejumomi *et al*., (2008) and earlier recorded that bitter leaf contains an alkaloid which is capable of reducing headaches associated with hypertension. Alkaloids are a diverse group of secondary metabolites found to have antimicrobial activity by inhibiting DNA topoisomerase (Han *et al*., 2007). Saponins, present in plants, have been suggested as possible anticarcinogens, saponins protect against hypercholesterolemia and antibiotic properties. These structurally diverse compounds have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoglycemia, and to act as antifungal and antiviral agents (Brown and Rice-Evans, 1998; Krings and Berger, 2001) .Tannins reduce the risk of coronary heart diseases (Just *et al*., 1998). Tannins may be employed medicinally in antidiarrheal, haemostatic, and antihemorroidal compounds. The anti-inflammatory effects of tannins helps to control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Diarrhea is also treated with an effective astringent medicine that does not stop the flow of the disturbing substance in the stomach; rather, it controls the irritation in the small intestine (Sodipo, 2000). The importance of alkaloids, saponins and tannins in various antibiotics used in treating common pathogenic strains has recently been reported by (Epand *et al*., 2007 and Okwu, 2001).Phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Okwu, 2001).A number of studies have focused on the biological activities of phenolic compounds, which are potential antioxidants and free radical scavengers (Del-Rio *et al*., 1997), phenols are involved in defense against UV radiation or aggression by pathogens. Consumption of diets rich in plant polyphenols offer protection against development of cancer, cardiovascular diseases, diabetes, osteoporosis and neurodegenerative diseases. Flavonoids are used to treat many important common diseases due to their proven ability to inhibit specific enzymes to stimulate some hormones and neurotransmitters and to scavenge free.

Medicinal plants are widely used in the management of various diseases including inflammation. Phytochemical analysis conducted on this plant extract revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. The preliminary phytochemical screening reveals the presence of some phytochemicals such us carbohydrate, alkaloids, cardiac glycoside, tannins, saponins, anthraquinone and flavonoids which was known to have anti-inflammation. The toxicity studies of this plant was happened to reveal the safety of the plant because even at 5000 mg/kg was safe. The anti-inflammatory effects of the methanol extracts of *A. uniflora* were evaluated by carrageenan-induced mice paw oedema method and the result was shown in Table 3. The extract were tested at three different doses levels such as 250, 500 and 1000 mg/kg.

The results with higher dose of the plant extract at 1000 mg/kg exhibits high activity with 65 % inhibition at 96 hr, followed by 250 mg/kg with 55 % and 500 mg/kg with 47 % indicating that a dose of 1000 mg/kg showed a maximum anti-inflammatory effects statistically as compared to the standard drug which had only 40 %. In general, oedema has an early stage of inflammation (Silva *et al.*, 2005) is due to release of histamine and serotonin like substances (Maridass and Ghanthi, 2008). Higher dose (1000 mg/kg) of *A. uniflora* extract anti-inflammatory activity may be due to inhibition of the mediators of inflammation histamine, serotonin and prostaglandin at after 96 hr.

CONCLUSION

The presence of these phytochemicals in this plant includes carbohydrates, cardiac glycoside, flavonoids, saponins, alkaloids, anthraquinone and tannins. The findings of this study suggests that this plant could be a potential source of natural anti-inflammatory drug that could have great importance as therapeutic agents in preventing various diseases hence it is safe. Further investigations on the chronic toxicity, isolation and characterization of the anti-inflammatory constituents is however required.

Contributions of Authors

Anas A. as main author conducted the research. A. A. Ambi and Zainab M. designed and supervised the work lastly Jajere U.M and Saifullahi U. performed statistical analysis.

Conflicts of Interest

No conflicts of interest.

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