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Determination of Phytochemical and Anti-Inflammatory Effects of Ethylacetate and N-Hexane *Extracts* of *Aeschynomene Uniflora*

[1]Anas, A.*,[2]Namadina M.M. & [2]Saifullahi, U.
[1&2]Department of Pharmacognosy and Herbal Medicine,
Bayero University Kano,
Kano State– Nigeria.
[2]Department of Plant Science,
Bayero University Kano,
Kano State- Nigeria.
Email: anasringim@yahoo.co.uk

Abstract
Aeschynomene uniflora studies was carried out with the aim to validate the scientific claims by evaluating the phytochemical constituents, toxicity and anti-inflammatory effects of both the Ethyl acetate and n-Hexane extracts. 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 extracts. Toxicity studies by using oral route showed no death in any group of the animals, even at 5000 mg/kg indicating its safety. Acute inflammation studies revealed the reduction of paw edema that was significant ($p< 0.05$) at dose of 500 mg/kg when comparing the treated groups with distilled water in the case of ethyl acetate extract as seen in table: 3 with the percentage of inhibition of 42.90 % when compared with the standard drug that has 58.33 %. With regard to the n-hexane extract. There was significance at 1000 mg/kg from the range of 0.5 hr to 4 hr when comparing the treated groups with distilled water and also the standard drug with the percentage of inhibition of 50.23 % and 31.11 % respectively. The phytochemicals obtained from 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. And this supports the ethnomedical claim of the use of the plant in management of inflammatory condition especially when using the nonpolar menstrum.

Keywords: Aeschynomene uniflora, Anti-inflammatory, Phytochemicals, Toxicity

INTRODUCTION
Medicinal plants are used by 80% of the world population for their basic health needs. There has been an increasing interest in the study of medicinal plants as natural products in different parts of the world (Panchavarnakili *et al*., 2012). Medicinal plants represent rich sources of antimicrobial agents used medicinally in different countries and are a source of many potent drugs used for traditional medicine. They contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Newman and Cragg, 2012). Medicinal plants play important role in meeting the basic health needs in many developing countries and the

*Author for Correspondence

industrialized countries. The use of medicinal plants has been based on the extraction which has led to the development of several drugs traditionally used in folk medicine (Lifongo *et al*., 2014). Various drugs with medicinal properties have been attributed to natural herbs and medicinal plants which constitute the main source of new pharmaceuticals and health-care products (Savithramma *et al*., 2011). *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, 2000). 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).

MATERIALS AND METHODS

Collection and Identification of Plant material

Plant specimen collection were collected 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 of both Ethyl acetate and n-Hexane. The contents were then be filtered using 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 both Ethyl acetate and Hexaneextracts according to (Abimbola *et al*., 2013; Evans, 2009) as outlined below.

Alkaloids

Dragendorff's test; to 2 mg of both ethyl acetate and n-hexane extracts 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 was added. Formation of orange or orange red precipitate indicates the presence of alkaloids.

Wagner's test; 2 mg of both ethyl acetate and hexane extracts 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 both ethyl acetate and n-hexane extractswere added. Formation of white or pale yellow precipitate indicates the presence of alkaloids.

Flavonoids
Shinoda's test; 2 mg of both ethyl acetate and hexane extractswere 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 extracts 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 both ethyl acetate and hexane extracts, a drop of sodium bicarbonatesolution 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 extracts 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 extracts was shaken with chloroform, to the chloroform layer sulphuric acid were added slowly by the sides of test tube. Formation of red colour indicates the presence of steroids.

Tannins
To 1-2 mg of both ethyl acetate and hexane extracts, few drops of 5% w/v $FeCl_3$ solution was added. A greencolour indicated the presence of gallotannins, while brown colour indicates the presence ofpseudotannins.

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 both the ethyl acetate and hexane extract were dissolved in 10 ml of distilled water 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 were grouped in to 3 by 3 groups and the dose administered for each group of mice were then calculated using the formula; Dose × Weight of mice in kg/ stock solution.
- The extracts were 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 were 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 extracts were carried out as described above.
- The acute toxicity was then calculated using the formular below.

$$\sqrt{\text{minimum dose of death} \times \text{minimum dose of survival}}$$

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

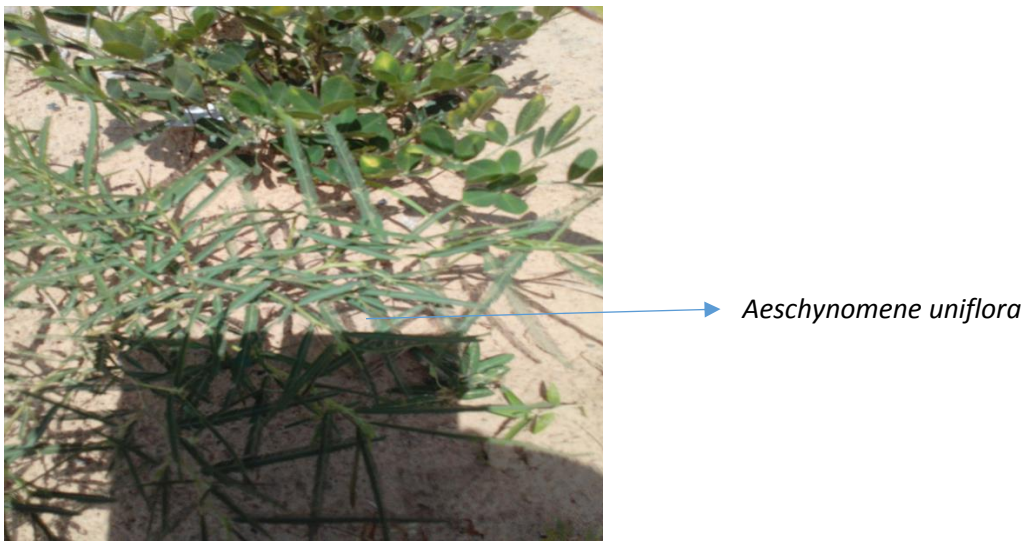
Freshly prepared 1% carrageenan or textran in 0.1 % carboxy methyl cellulose (0.02 ml) was injected on subplantar region of the right paw of mice to induce acute inflammation (Winter *et al*., 1962 and Maity *et al*., 1998) with some modifications. The animals were divided in to 8 groups, 6 animals each. Group I served as control for carrageenan. Group II and III received 250 mg/kg and 500 mg/kg body weight extracts (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 Vernier 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 mg/kg, 500mg/kg and 250 mg/kg to see how effective were the extracts was, at lower, middle and the high doses and also the animals were divided in to five groups, 5 animals each, hence our extracts toxicity studieswere practically non-toxic because even at 5000 mg/kg were no death encountered.Percentage of inhibition were calculated using formula below;

$$\%\text{inhibition of paw thickness} = \left[\frac{(tCn - tC0) - (tTn - tT0)}{(tCn - tC0}\right] \times 100.$$

STATISTICAL ANALYSIS

All data were 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



Aeschynomene uniflora inits natural Habitat

Table 1: Phytochemical constituents from both Ethylacetate and n-Hexane Extracts of *Aeschynomene uniflora*

Test Constituents	Ethyl acetate	n-Hexane
Carbohydrate	+	+
Cardiac glycoside	+	-
Tannins	+	-
Saponinns	+	+
Flavonids	+	-
Anthraquinone	+	-
Steroid	-	+
Triterpenes	-	+
Alkaloid	+	-

+ = present, - = absent

Acute Toxicity Study

The acute toxicity studies of the extracts gave no LD_{50} even at 5000 mg/kg in mice, using oral route of administration. Results was presented in the table 2.

Table 2: Acute Toxicity Study of both Ethylacetate n-HexaneExtracts 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: Anti-inflammatory effects of the Ethyl acetate Extract

Treatment	Dose (mg/kg)	At 30 minutes	At 1 hour	At 2 hours	At 3 hours	At 4 hours	At 5 hours
Control	D. Water	0.430±0.040	0.488±0.033	0.638±0.060	0.758±0.038	0.752±0.038	0.648±0.068
Aeschynomene uniflora	1000 mg/kg	0.424±0.034	0.540±0.071	0.786±0.071	0.640±0.015	0.638±0.037	0.404±0.028
Ethyl acetate Extract	500 mg/kg	0.342±0.056	0.058±0.101	0.592±0.074	0.622±0.065	0.556±0.054*	0.370±0.036*
	250 mg/kg	0.388±0.038	0.466±0.090	0.612±0.030	0.738±0.032	0.576±0.039*	0.414±0.050*
Diclofenac	10 mg/kg	0.296±0.044	0.338±0.048	0.434±0.039	0.422±0.032*	0.386±0.039*	0.270±0.033*

n= 5, *significance at P<0.05 when each groups were compared with distilled water, D= Distilled water. Dunnett t-tests treat one group as a control, and compare all other groups against it.

Table 4: Anti-inflammatory effects of the n-Hexane Extract

Treatment	Dose (mg/kg)	At 30 minutes	At 1 hour	At 2 hours	At 3 hours	At 4 hours	At 5 hours
Control	D. Water	0.430±0.040	0.488±0.033	0.638±0.060	0.758±0.038	0.752±0.039	0.648±0.068
Aeschynomene uniflora *Ethyl acetate Extract*	1000 mg/kg	0.214±0.034**	0.304±0.042**	0.378±0.029**	0.528±0.042	0.446±0.040	0.348±0.047
	500 mg/kg	0.300±0.049	0.362±0.052	0.538±0.082	0.608±0.038	0.564±0.068	0.500±0.063
	250 mg/kg	0.262±0.043*	0.356±0.036	0.452±0.054	0.630±0.060	0.412±0.033*	0.416±0.054
Diclofenac	10 mg/kg	0.296±0.044	0.338±0.048	0.434±0.039	0.422±0.032*	0.386±0.039	0.270±0.033*

n= 5, ** highly significance at P<0.05, *Significance at P<0.05when each groups were compared with distilled water, D= Distilled water. Dunnett t-tests treat one group as a control, and compare all other groups against it.

DISCUSSION

Plants have the ability to produce a large varieties of secondary metabolites such as saponins, tannins, phenols, alkaloids, triterpens and phytosterols that can protect against chronic diseases. 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; 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).

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). 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 acute anti-inflammatory effects of both the ethyl acetate and hexane extracts of *A. uniflora* were evaluated by carrageenan-induced paw oedema method and the result was shown in Table 3 and 4respectively. The ethyl acetate extract were tested at three different doses levels such as 250 mg/kg, 500 mg/kg and 1000 mg/kg.

Acute inflammation revealed the reduction of paw edema that was significant ($p< 0.05$) at dose of 500 mg/kg when comparing the treated groups with distilled water in the case of ethyl acetate extract as seen in table: 3 with the percentage of inhibition of 42.90 % when compared with the standard drug that has 58.33 %. With regard to the hexane extract, significance ($p< 0.05$) occurred at 1000 mg/kg from the range of 0.5 hr t 4 hr when comparing the treated groups with distilled water and also the standard drug with the percentage of inhibition of 50.23 % and 31.11 % respectively. Carrageenan is widely used as phlogistic agent in order to test anti-inflammatory drugs (Vogel, 2008; Aiyelero *et al.,* 2009, Ismail *et al.,* 2015). The formation of edema that was induced by carrageenan was known to be in two phases; the first or early phase i.e the first hour after carrageenan injection involves the release of serotonin, histamine and bradykinin; while the second or late phase i.e 2–4 hours after carrageenan injection may be associated with increased edema formation that remains up to the fifth hour and involves the release of prostaglandins and lysosomal enzymes (Ratheesh and Helen, 2007; Khan *et al.,* 2009).

CONCLUSION

The phytochemicals obtained from 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. And this supports the ethnomedical claim of the use of the plant in management of inflammatory condition especially when using the nonpolar menstrum.

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