Albizia zygia (D.C.) Macbr (Fabaceae): A Comparative Investigation of Phytochemical Composition, Proximate Analysis and Anti- Seizure Properties of Methanol Extracts of Its Leaves and Stem-Bark.

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ABSTRACT

Albizia zygia belongs to the legume subfamily Mimosoideae. Albizia lebbecka, a congener of *Albizia zygia* is reported to possess anti-seizure activity and folk reports of sedation when the leaves of *Albizia zygia* are consumed. This aim of this study was to compare the phytochemistry, proximate parameters and possible anti-seizure properties of the methanol extracts of the leaves and stem-bark of *Albizia zygia*. Phytochemical screening and proximate analysis were carried out according to standard methods. Pentylentetrazol (PTZ) and maximum electroshock (MES) test were employed to investigate the anti-seizure properties in mice at 200, 400 and 800 mg/kg doses. Both samples had saponins and flavonoids in common but phenols were only present in the leaves while alkaloids, tannins and proteins were found only in the stem-bark. Anti-seizure activity of the stem-bark was superior to that of the leaves and was significant ($p<0.05$) in the PTZ test. This study lends support to the ethnomedicinal use of the plant in the management of seizures.

Keywords: *Albizia zygia*; Methanol extract; Phytochemistry; Anti-seizure

INTRODUCTION

Albizia zygia, is a member of the legume subfamily Mimosoideae. The genus Albizia comprises more than 150 species, mostly trees and shrubs native to tropical and subtropical regions of Asia and Africa (Orwa *et al*, 2009; Odeyemi *et al*., 2014). Albizia species are used in folk medicine for the treatment of rheumatism, stomach ache, cough, diarrhoea, wounds and anthelmintic. Different parts of the plant have been used ethno-medicinally. For example, the bark is used in southern Sudan as powder or decoctions to treat malaria. The bark sap is instilled in the eyes to treat ophthalmia. A bark decoction is administered to treat bronchial diseases, fever, female sterility, purgative, stomachic, antidote, vermifuge and aphrodisiac. Pounded or rasped bark is applied externally to treat yaws, sores, wounds and toothache. The grounded roots are added to food to treat cough and as an expectorant; brain, nervous system; general healing; pulmonary troubles; vermifuges; antidotes to venomous stings and bites. Leaf decoctions are used to treat fever, dysentery, diarrhoea, insanity and as pain killers (Burkill 1995). In traditional Indian and Chinese medicine, Albizia plants are used therapeutically for insomnia, irritability, wounds, antidysentric, antiseptic, antituberculosis. Phytochemical studies on the genus Albizia shows that they are a source of different group of natural products such as triterpenoids, saponins, diterpenoids, lignans and pyridine glycosides (Karuppannan *et al*; 2013). Evaluated pharmacological activities of Albizia species include: antioxidant (Khartoon *et al*., 2013, Steinrut et al., 2011), anticancer (Zheng *et al*, 2006, Miyase *et al*., 2010), analgesic (Lau *et al* 2007; Melek *et al*; 2007; Zou *et al.,* 2006), anti-inflammatory, (Karuppannan *et al*., 2013; Siju *et al*., 2014), antidiabetic (Kang et al., 2000; Margaret and Jaykar 2013), antibacterial (Sarkiyayi *et al*., 2011), hepato-protective (Mar et al., 2012), antihyperlipidemic (Saraj *et al*., 2012) and diuretic (Sivakumar *et al*., 2013), sedative and anticonvulsant (Grade *et al*., 2008), antimalarial and antiprotozoan (Ndjakou *et al*., 2009, Muna *et al*., 2012), analgesic (Abere *et al*., 2014), antibacterial (Odeyemi *et al*., 2014).

Pharmaceutically, *A. zygia* gum has been shown to have good suspending properties (Mbang *et al*., 2014). This present study was embarked on to evaluate comparatively: the phytochemistry, proximate parameters and anti-seizure properties of the methanol extracts of the leaves and stem-bark of *Albizia zygia*.

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MATERIALS AND METHOD

Sample collection and preparation of plant materials

The leaves and stem-bark of *Albizia zygia* were collected from Tenboga community in Uhumwonde Local Government Area of Edo state, in July 2016. The plant was identified and authenticated by Prof. B.A Ayinde of the department Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria and assigned voucher No UBHp 0312. The collected plant parts were air dried under shade (leaves for 2 weeks and stem bark for 4 weeks). The air dried samples were thereafter ground to powder using an electric mill with model No OWBL440A06 designed and engineered by Kenwood in the UK, made in China. The powdered samples were weighed and stored in an air tight container prior to use.

Phytochemical screening and extraction

Phytochemical screening and proximate analysis were carried out on the dried powdered leaves and stem-bark of *Albizia zygia* according to standard procedures (Evans 2005).

Extraction of plant material

Powdered stem-bark or leaves of *Albizia zygia* (800 g) was extracted with 2 L of methanol by maceration at room temperature for 48 hours. The extracts were concentrated to dryness using a rotary evaporator at reduced pressure. The concentrated extracts were weighed and the percentage yield calculated. The extract was stored in air tight containers in a refrigerator at 4^{o}C until further experiment.

Test for Anticonvulsant Activity of The Leaf of *Albizia zygia*

Animals

Swiss albino mice weighing between 20-30 g of both sexes (pregnant females excluded) were used. The experimental protocol was approved by institutional Animal Ethical Committee (Ref No UBE/2016/054) to carry out and complete the study. The animals were allowed to acclimatize, allowed free access to food, water and 12:12 hour day and night time. The animals were starved overnight but allowed access to fresh water before commencement of biological study. The animals were divided into groups of five animals each for the experiment.

Pentylenetetrazole (PTZ) Induced Convulsion in Mice

All drugs were administered orally by gavage. Forty (40) animals (mice) were used and they were divided into eight groups (five animals per group). The control group was administered distilled water (2 ml/kg), to another six groups (test groups) was administered either the methanol extract of the leaves or stem bark of *Albizia zygia* at 200 mg/kg, 400 mg/kg and 800 mg/kg doses suspended in distilled water. The last group received 40 mg/kg Phenobarbitone intraperitoneally and served as the standard group. One hour after administration, each mouse was administered pentylenetetrazole (PTZ) 70 mg/kg subcutaneously (SC). The mice were observed for one hour after administration of PTZ for seizure and time of death. The time for the animal to exhibit seizure and time of death were recorded. Any mouse that showed neither convulsion nor death was considered protected.

Maximum Electroshock (MES) Induced Convulsion in Mice.

Control, test extracts and reference drug were administered as in the PTZ model above. One hour after administration, a current of 50 mA from a standard current supplier was applied to each mouse through a pair of ear-clip electrodes for 0.2 seconds.

STATISTICAL ANALYSIS

All results are expressed as Means ± S.E.M. The result was analyzed by one way ANOVA followed by t-test using the Statistical Analysis System (SPSS Statistics 17.0). The p-value was set at 95%.

RESULTS AND DISCUSSION

Proximate analysis

The methanol extractive, water soluble extractive and calcium content were higher for the leaves than for the stem-bark sample whereas total ash, acid insoluble ash were higher in the stem-bark sample as shown in table 1.

Table 1: Proximate parameters of leaves and stem-bark of *Albizia zygia*

TEST	RESULTS LEAVES	STEM BARK
Yield of extract (%)	4.53	0.94
Moisture content (%)	11.67± 1.36	33.33± 2.47
Ash Values		
Total ash (%)	4.99±0.07	5.43±0.55
Acid insoluble ash (%)	1.50±0.21	3.75±0.68
Water soluble ash (%)	0.05±0.00	2.14±1.13
Extractable matter		
Alcohol soluble (%)	11.43±1.04	0.69±0.05
Water soluble (%)	8.05±0.54	0.08±0.04
Mineral composition		
Calcium (mg/kg)	42.14±1.21	23.50±1.34
Magnesium (mg/kg)	3.93±0.25	20.51±2.31

Phytochemical analysis

Carbohydrates (but not reducing sugars), saponins (including steroidal saponins) and flavonoids were present in both the dried leaves and stem-bark. Whereas phenolics were only present in the dried leaves, alkaloids, tannins and proteins were only present in the stem-bark as shown on table 2.

Table 2: Qualitative Phytochemical Analysis of the Powdered Leaves and stem-bark of *Albizia zygia*

TEST	RESULTS	
	LEAVES	STEM-BARK
Alkaloids	_	+
Carbohydrate	+	+
Reducing sugars	-	_
Saponins	+	+
Steroidal saponins	+	+
Tannins	_	+
Phenolics	+	_
Flavonoids	+	+
Anthraquinones	_	_
Protein	_	+

+ = present, - = absent

Anti-seizure activity

Anti- seizure activity of MLE and MES of *A. zygia* are shown on table 3. The anti-seizure activity of the MLE was found to be highest at the dose of 200 mg/kg by delaying time of death. The MSE showed a dose dependent anti-seizure activity delaying the onset of seizures, time of death as well as reduced mortality of the animals in the PTZ induced seizures. However, both the extract samples did not protect the animals against the MES induced seizures at all doses. Phenobarbitone (40 mg/kg) protected the animals against both PTZ and MES induced seizures.

Table 3: Anti-seizure activity of the methanol leaf extract (MLE) and stem-bark extract (MSE) of *Albizia zygia*

Drugs	Dose (mg/kg)	Anticonvulsant **PTZ** Onset of Seizures (Sec) (Mean ±SEM)	Activity **TEST** Time of death (Sec) (Mean ±SEM)	(% Mortality) **MES Test**
MLE (p.o)	200	67.00±3.40	148.40±5.15(20)	80 (NP)
	400	51.80±3.40	69.83±3.35(80)	100 (NP)
	800	90.60±2.08	92.40±2.98(60)	80 (NP)
MSE (p.o)	200	74.84±0.00	114.44±0.00(20) **	80 (NP)
	400	257.00±34.09**	286.41±35.6(100) **	80 (NP)
	800	217.00±95.26**	301.43±103.8(80) **	80 (NP)
Phenobarbitone (i.p)	40	634.59±32.71**	658.83±33.35(100) **	0 (P)
Distilled water (p.o)	2 ml/kg	39.20± 1.55	73.25±2.05	100 (NP)

MLE = Methanol Leave Extract, **MSE** = Methanol Stem-bark Extract, ***p.o*** = *per oral*, ***i.p*** = *intraperitoneal*, **NP** = Not Protected, P = Protected.
**indicates significantly (*p<0.05*) different anti-seizure activity.

DISCUSSION

Odeyemi et al 2014 reported the presence of alkaloids, flavonoids, tannins, saponins and cardiac glycosides in the leaves of *Albizia zygia* and Abere *et al* in 2014 reported the presence of alkaloids, tannins, flavonoids and saponins. From the results on table 2, the leaves and the stem-bark samples have saponins (particularly steroidal) and flavonoids in common. However, while alkaloids, tannins and proteins were found only in the stem-bark, phenolics were only present in the leaves. This goes to show that secondary plant metabolites are not evenly distributed among plant parts. In table 1, results indicated almost three times higher moisture content for the stem-bark than for the leaves possibly because of the presence of the gum produced by the bark of *Albizia zygia* which is of pharmaceutical useful as a suspending agent (Mbang *et al*., 2014). Both samples had higher than required moisture content (6-8%) for crude natural products (African Pharmacopoeia 1986). As a result, proper storage conditions to prevent possible degradation should be ensured for these samples. From the extractable matter results the preferred extractive solvent for both samples was alcohol, however, the leaves gave better yield as compared to the stem bark. Ash values which are an indication of the organic and inorganic content of samples generally showed that the stem-bark had higher values than the leaves.

Medicinal plants derive their physiological and pharmacological activities from the presence of different bioactive constituents' mostly secondary metabolites they contain or express (Sofowora, 1993) and these metabolites may or may not be distributed evenly in different parts of the same plant (Achakzai *et al*., 2009) and could be the reason for the different activities exhibited by different plant parts. From the antiseizure screening results (table 3), the methanol Stem-bark Extract (MSE) showed generally significant ($p < 0.05$) antiseizure activity in both the onset of seizures and time of death in the PTZ assay (Petit mal model) as compared to the leaves (MLE). In the MES assay which mimics Grand-mal seizures both MLE and MSE samples failed to protect the animals at the doses employed in this present study. Phenobarbitone (reference drug) however, exhibited superior antiseizure activity in both the PTZ and MES assays than the test samples.

CONCLUSION

This study has shown comparative differences in the phytochemical composition and some proximate parameters of the leaves and stem-bark of *Albizia zygia*. The difference in possible antiseizure activity has also been established and the differences in the composition of the dried leaves and stem-bark of Albizia zygia may be responsible for the differences in antiseizure activity exhibited by the different parts. Further studies are needed to identify the active constituents.

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