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Preliminary Phytochemical and Acute Toxicity Studies of Methanol Leaf Extract of *Acioa Barteri*

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Abstract

Acioa barteri plant has higher medicinal value and can be extensively studied to extract the natural compounds which are beneficial to human beings and that could be commercialized for higher production than using synthetic drugs with side effects. Plants have served human beings as a natural source for treatments and therapies from ancient times. Medicinal plants have gained attention because of its wide use and less side effects. We investigated the phytochemical constituents and acute toxicity of leaves extract of *Acioa barteri*. The presence of tannins, saponins, terpenoids, flavonoids, alkaloid, phenol, steroids and cardiac glycosides were determined using standard methods for qualitative phytochemical analysis. A total number of 12 rats were used for acute toxicity studies; grouped into 4 groups of 3 albino mice each. The plant extract was administered according to their body weights with 250, 500 and 1000mg/kg. The plant extract at 250mg/kg and 500mg/kg showed no significant ($p<0.05$) toxic effects while at higher dose of 1000mg/kg, the extract caused mortality of experimental animal. The oral lethal medium dose (LD50) of 707.15mg/kg was recorded. The treatment of the mice with different doses of the plant extract (250, 500, and 1000mg/kg) significantly altered the serum marker enzymes (AST, ASP and ALT). From the study, the extract is safe on acute administration but prolong use may cause harmful effect on the animal organs. The phytochemical studies and acute toxicity of the plant leaves were evaluated for the first time, hence a good plant to explore for its medicinal potentials.

Keywords: *Acioa barteri*; Phytochemical constituents; Acute toxicity; Medicinal plants; Albino mice

Abbreviations: ALP: Alkaline Phosphate; AST: Aspertate Transaminase; ALT: Alanine Transminase.

Introduction

Nigeria is blessed with many plants for ethnomedicinal use. Acioa barteri is rich in phytochemicals. Because of its bioactive constituents, it is used traditionally for the treatment of various diseases and as animal feed. Bioactive constituents in medicinal plants are responsible for their therapeutic actions [1]. Medicinal plants have been traditionally used in different kinds of ailments including infectious diseases, which account for approximately one-half of all deaths in tropical countries [2]. Plants are rich in wide variety of secondary metabolites, such as tannins, saponins,

terpenoids, alkaloids and flavonoids which have been found in vitro to have antimicrobial properties. Historically, plants have provided a good source of anti-infective agents. The higher plants, for example, Acioa barteri will make important contributions in areas beyond anti-infective such as cancer therapies. Scientists from different fields are investigating plants with an intention to discover valuable phytochemicals. Laboratories all over the world have also found literally thousands of phytochemicals which have inhibitory effects on all types of microorganisms in vitro [3].

Pharmacological actions of medicinal plants include; antioxidant, antimalaria, antidiabetic, antidiarrhea, antitumoral or anticancer, antioxidant, diuretic, analgesic, antimicrobial, anti-inflammatory antibacterial, antifungal, hepatoprotective, immune-modulatory, laxative and sedative activities etcetera. Extracts from leaves, stems, roots, flowers and seeds of *Acioa barteri* are used locally to prevent ailments. According to the ethnic difference of populations and localities, medicinal plants are either used alone or in combination with other plants or with natural substances for the preparation, especially in decoction [4]. Oral route of administration is mostly used for this preparation. Medicinal plants also help in the alleviation of human suffering especially amongst those living in rural areas.

Herbal medicine has a long history in the treatment of several kinds of diseases [5]. The use of medicinal plants for the treatment of diseases has been practiced by man for many years and is still being widely practiced even today [6]. For many years, people have developed a store of information concerning the therapeutic values of local plants before orthodox medical practice appeared. These herbalist and their apprentices through periods of trials and error and success in some cases, have accumulated a large body of knowledge about medicinal plants.

According to Iwu MM, et al. [2], the first generation of plant drugs were usually simple botanicals employed in more or less their crude form. Several effective medicines used in their natural state were selected as therapeutic agents based on empirical studies of their applications by traditional society from different parts of the world. Plant materials remain an important component in combating serious diseases in the world, for their therapeutic approaches to several pathologies. Following the industrial revolution, a second generation of plant drugs emerged based on scientific processing of the plant extract to isolate their active compounds. Interest in medicinal plants has been over whelming in the recent times, especially as an important source of medication and health care. Medicinal plants have been globally recognized to have played a significant role in providing health benefits to human being.

The World Health Organization [7] has estimated that 80% of the inhabitants of the world rely mainly on the traditional medicine for their primary health care needs and it may be presumed that a major part of traditional healing involves the use of plant extracts or their active principles. The effect of methanol extract of *Acioa barteri* on hepatocellular damage and lipid profile of Albino rat has be investigated [8].

Methodology

Plant Collection and Identification

Fresh leaves of *Acioa barteri* plants were collected from a farm in Amudi village in Ezinihitte Mbaise in Imo State, Nigeria. The leaves was identified and authenticated by Dr. Garuba Omosun of the Department of Plant Science and Biotechnology Michael Okpara University of Agriculture Umudike, Abia State Nigeria.

Sample Preparation and Extraction

The cleaned, healthy plant leaves were washed, dried at room temperature and pulverized. About 1500g of the pulverized leaves was subjected for extraction of active constituents by cold maceration using methanol as the solvent. The extraction was allowed to stand for 72 hours (3 days) with slight and timely shaking. The resultant mixture was first filtered through muslin cloth and subsequently filtered with Whatman (No. 1) filter paper. The filtrate was then concentrated using a rotary evaporator maintained at temperature 40^0-50^0C to obtain a constant weight of the extract.

The extract was weighed and was re-dissolved in the solvent to get a final concentration 1mg/ml. The percentage yield of the dried residue was calculated. The dried extract was stored in a refrigerator awaiting administration and phytochemical analysis.

Experimental Animals

Healthy albino mice (130-1180g) were used for the study. The animals were obtained from Animal house of Department of Pharmacology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Anambra State and housed under normal room conditions for two (2) weeks to acclimatize. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals. Animals were handled in compliance with ARRIVE guidelines [9], while the experiments were conducted according to standard protocol of National Institute of Health (NIH) [10] guidelines for use and care of laboratory animals.

Acute Toxicity Studies

Twelve albino mice were used for the study. The animals were randomized according to their weights and kept in four stainless steel cages containing 3 mice each. Test Groups were given 250, 500 and 1000mg/kg body weight respectively of methanol leaf extract of *A. barteri*. Group four represented the normal control that did not receive the extract, but food and water only. The different groups were examined for change and behavior (toxic effect) after 24 and 48 hours, and then the observation and the number of deaths were recorded.

Phytochemical Analysis

Qualitative Analysis (Preliminary Screening)

The phytochemical screening of the leaves extract was carried out by standard procedures described by Harborne [11], Trease and Evans [12] and Sofowara [13].

Test for flavonoids: 2 ml of the extract was added with few drops of 20% sodium hydroxide, formation of intense yellow colour is observed.

To this, few drops of 70% dilute hydrochloric acid were added and yellow colour was disappeared. Formation and disappearance of yellow colour indicates the presence of flavonoids in the sample extract.

Test for alkaloids: To 1 ml of the extract, 1 ml of marquis reagent, 2ml of concentrated sulphuric acid and few drops of 40% formaldehyde were added and mixed, appearance of dark orange or purple colour indicates the presence of alkaloids.

Test for saponins: To 2 ml of the extract, 6 ml of distilled water were added and shaken vigorously; formation of bubbles or persistent foam indicates the presence of saponins.

Test for Tannins: To 2 ml of the extract, 10% of alcoholic ferric chloride was added; formation of brownish blue or black colour indicates the presence of tannins.

Test for phenols: To 2 ml of the extract, 2 ml of 5% aqueous ferric chloride were added. Formation of blue colour indicates the presence of phenols in the sample extract.

Test for Proteins: To 2 ml of the extract, 1 ml of 40% sodium hydroxide and few drops of 1% copper sulphate were added. Formation of violet colour indicates the presence of peptide linkage molecules in the sample extract.

Test for cardiac glycosides: 1 ml of the extract was taken, 0.5ml of glacial acetic acid and 3 drops of 1% aqueous ferric chloride solution were added.

Formation of brown ring at the interface indicates the presence of cardiac glycosides in the sample extract.

Test for terpenoids: 1 ml of extract of the solvent was taken and 0.5 ml of chloroform was added followed by a few drops of concentrated sulphuric acid, formation of reddish brown precipitate indicates the presence of terpenoids in the extract.

Statistical Analysis

Results were analyzed using One Way ANOVA (FischerLSD post hoc test) and expressed as mean±SD. Difference between Means of treated and control groups were considered significant at $P< 0.05$.

Results

The results below show the acute toxicity studies for three different groups of laboratory animal

Groups	250mg/kg	Number of death
Control	16.56±2.26[a]	0
After 24hours	18.80±3.22[a]	0
After 48hours	16.92±1.04[a]	0

Table 1: Acute toxicity studies for group 1.

Values are expressed as mean standard deviation; [a]Anova indicated significantly ($p<0.05$) different from the control. (n= 3)
NB: Total number of death = 0

Groups	500mg/kg	Number of death
Control	18.58±2.48[a]	0
After 24hours	20.47±7.61[a]	0
After 48hours	20.08±8.18[a]	0

Table 2: Acute toxicity studies for group 2.
Values are expressed as mean standard deviation of 3 mice; [a]Anova indicated significantly ($p<0.05$) different from the control. (n= 3)
NB: Total number of death = 0

Groups	1000mg/kg	Number of death
Control	18.48±5.81[b]	0
After 24hours	0.00±0.00[a]	All (3)
After 48hours	0.00±0.00[a]	All (3)

Table 3: Acute toxicity studies for group 3.
Values are expressed as mean standard deviation; [a,b]Anova indicated significantly ($p<0.05$) different from the control. (n= 3)
NB: Total number of death = 6

Phytochemicals	Inference
Alkaloids	+
Tannins	+++
Saponins	+
Flavonoids	++
Terpenoids	++
Steroids	++
Cardiac glycosides	++
Resins	+

Table 4: Results of qualitative analysis of phytochemicals present in methanol extract of *Acioa barteri.*
Keys: +++ = Very strong positive, ++ = Strong positive, + = Weak positive

Discussion

The phytochemicals are important to ascertain the medicinal values of *Acioa barteri*. The phytochemical screening shows the presence of alkaloids, tannins, saponins, flavonoids, terpenoid, resins and cardiac glycosides. The presence of these secondary metabolites suggests potentials for the plant as a source of important phytomedicines. Some plants that possess alkaloids are known for decreasing blood pressure and balancing the nervous system. Tannin as an astringent helps in wound healing and as an anti-parasitic. The presence of terpenoids suggests its use as anti-tumor and anti-viral agent. They are known to be cytotoxic to tumor cells. Alkaloids are known to possess anti-malaria property, so this plant may be a good source of antimalarial. Cardiac glycosides have been effective in the treatment of congestive heart failures, cancer and as anti-arrhythmia agent. Plants containing saponins and flavonoids are believed to have antioxidants, anti-cancer, antiinflammatory and anti-viral properties.

Acioa barteri seemed to be less toxic justifying from its wide use in folklore medicine. Indeed, the leaves extract (250mg/kg and 500mg/kg) showed no toxicity in experimental mice. After 24 hours and 48 hours of administration of the extract (250mg/kg and 500mg/kg) in group 1 and 2, there was no significant difference from the mass (weight) of the animals compared to control (Tables 1 & 2) respectively, but group 3 showed significant difference after administration of extract at dose of 1000mg/kg compared to control (Table 3).

However at a higher dose, 1000mg/kg, the methanol leaves extract initiated hypersensitivity, cytotoxicity and increased aggressiveness and eventually caused mortality of experimental albino mice. This toxicity involves a drastic reduction ($p<0.05$) in the activities of the alkaline phosphate (ALP), aspertate transaminase (AST) and Alanine transminase (ALT) in the liver (Table 4).

Conclusion

Considering the studies, it may be stated conclusively that the methanolic extract of *Acio barteri* will provide a new therapeutic avenue against disease conditions. Moreso, the leaves of *Acioa barteri* were found to contain the most phytoconstituents validating their traditional use in the treatment of various ailments. It is obvious however, that *Acioa barteri* will have pharmacological functions such as; antimalarial, antidiabetic, antioxidant, anti-tumor, anti-inflammatory, antimicrobial, anti-diarrheal, diuretic, anti-fungal, antibacterial, exetera. From the toxicity study, the plant is safe for human and animal use (food for animals), though at high doses precaution should be taking.

Recommendation

Further studies are required to exploit the biomedical application of Acioa barteri. Studies should also be conducted to isolate, identify, characterize and elucidate the structures of these bioactive compounds.

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Conflicts of Interest

The author declares no conflict of interest

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