

Pharmacognostic studies, Phytochemical and Elemental Analysis of *Ipomoea Asarifolia* Leaves

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Abstract

Ipomoea asarifolia have been used for the treatment of various ailments such as diabetes, neuralgia, stomach ache and arthritic pain, dysmenorrhea, guinea sores and liver diseases. The study was aimed at determining the pharmacognostic, phytochemicals, elements and toxicity of the plant leaves. Findings from this study revealed the presence of some diagnostic microscopical features such as calcium oxalate, starch, gum/mucilage, lignin, Aleurone grain, suberized/Cuticular cell wall and inulin but calcium carbonate was absent. Quantitative physical constants include moisture contents (5.22%), ash value (10.89 %), acid insoluble ash value (3.17%), water and ethanol extractive indices 16.33% and 25.68% respectively.. Trace metals such as Fe (6.10ppm), Mn (3.85ppm) and Ni (0.43ppm) detected in *I. asarifolia* were below the permissible limit for edible plants while Pb (0.47ppm), Zn (2.10ppm), Cd (0.001ppm) and Cu (0.20ppm) were found to be within the limit. The phytochemical screening of the aqueous and methanolic extract of the leaves contain alkaloid, tannins, saponins, glycosides, steroids, phenols, carbohydrate, flavonoids and terpenoids. The LD₅₀ of the methanolic leaf extract of *I. asarifolia* was found to be greater than 5000 mg /kg and is considered safe for use. Nonetheless, further studies are encouraged to evaluate toxicity at much higher doses.

Keywords: Elemental analysis, *Ipomoea asarifolia*, Pharmacognostic, Phytochemical

Introduction

Ipomoea asarifolia which belongs to the Family Convolvulaceae (Nacro and Millogo-Rasolodimbi, 1993) is a creeping, glabrous succulent and perennial plant growing in waste land or sandy soil, river banks streams, low lying and inland valleys (Jegade *et al.*, 2009). According to Judd *et al.*, (1999), in Senegal, *I. asarifolia* is used traditionally to treat various gynecological ailments (including urinary problems during pregnancy, hemorrhage, abortifacient and ecboic). Similarly, the plant is used to treat arthritis pain, neuralgia, headache, wound dressing, ophthalmia (Aliyu *et al.*, 2011).

In Nigeria it is traditionally known as 'Duman-kaada', 'Duman-raafi' or 'Duman-kadu' in Hausa (northern Nigeria), 'Gboro-ayaba', 'Ododo-oko' and 'Ododo-amu' in Yoruba (south west-Nigeria) (Jegade *et al.*, 2009) and it have been used for the treatment of various ailments such as diabetes, neuralgia, stomach ache and arthritic pain, dysmenorrhea, guinea sores and

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liver diseases (Jegade *et al.*, 2009; Akindale *et al.*, 2014). Hausa/fulani people from northern Nigeria uses this herb to treat feverish chills and rheumatic pains, guinea-worm sores, syphilis (Dalziel, 1937). *Ipomoea asarifolia* or in combinations of other herbs is used in northern Nigeria to manage cancer disease (oral conservation, 2016).

Materials and Methods

Study Area

The study was conducted at Pharmacognosy Department, Ahamdu Bello University, Zaria, Nigeria.

Collection and Identification of *I. asarifolia* leaves

Fresh leaves of *I. asarifolia* were collected from local farm at Kumbotso Local government area, Kano state, Nigeria. The leaves were taken to the Herbarium section of Department of Plant Biology, Bayero University, Kano for identification.



Fig 1: *I. asarifolia* plants

Chemo-microscopic Studies of *I. asarifolia* powdered leaves

Powdered sample of *I. asarifolia* leaves was used for this study to detect the presence of cell wall materials and cell inclusions. Using various detecting reagents the presence of cell wall materials and cell inclusions was detected in accordance to WHO (2011) guidelines.

Test for Cellulose, Lignin, Suberized or Cuticular cell walls, Gum and mucilage and Cell Inclusions/ Cell Contents were determined by method of WHO (2011).

Determination of Physicochemical parameters of *I. asarifolia* powdered leaves

Some physicochemical parameters of the powdered sample of the plant such as moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol and water extractive values was determined as described in the updated edition of quality control methods for medicinal plant materials (WHO, 2011).

Qualitative Phytochemical screening of aqueous and methanolic leaf extract of *I. asarifolia*

The plant extracts (aqueous and methanol) were subjected to phytochemical screening in order to identify the phytochemical constituents of the plant using the method described below.

Tests for carbohydrates

- ❖ **Molish's (General) Test for Carbohydrates:** To 1 ml of the filtrate, 1 ml of Molish's reagent was added in a test tube, followed by 1 ml of concentrated sulphuric acid down the test tube to form a lower layer. A reddish colour at the interfacial ring indicates the presence of carbohydrate (Evans, 2009).

Tests for Saponin

- ❖ **Frothing test**

About 10ml of distilled water was added to a portion of the extract and was shaken vigorously for 30seconds. The tube was allowed to stand in a vertical position and was observed for 30mins. A honeycomb froth that persists for 10-15mins indicates presence of saponin (Evan, 1996).

Test for Flavonoids

- ❖ **Shinoda Test**

A portion of the extract was dissolved in 1-2ml of 50% methanol in the heat metallic magnesium chips and few drops of concentrated hydrochloric acid were added. Appearance of red color indicates presence of flavonoids (Evan, 1996).

Test for Alkaloid

- ❖ **Wagner's Test**

Few drops of Wagner's reagents were added to a portion of the extract, whitish precipitate indicates the presence of alkaloid (Evans, 1996).

Test for Steroid and Triterpenes

- ❖ **Liebermann-Burchard's test**

To a portion of the extract, equal volume of acetic acid anhydride was added and mixed gently. 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer. A colour change observed immediately and later indicates the presence of steroid and triterpenes. Red, pink or purple colour indicates the presence of Triterpenes while blue or blue green indicates steroids (Trease and Evans, 1996).

Test for Cardiac Glycoside

- ❖ **Kella-killiani's test**

A portion of the extract was dissolved in 1ml of glacial acetic acid containing traces of ferric chloride solution. This was then transferred into a dry test tube and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Observed carefully at the interphase for purple-brown ring, this indicates the presence of deoxy sugars and pale green colour in the upper acetic acid layer indicates the presence of cardiac glycosides (Evans, 1996).

Test for Tannins

❖ Ferric chloride test

To a portion of the extract, 3-5 drops of ferric chloride was added. A greenish black precipitate indicates presence of condensed tannins while hydrolysable tannins give a blue or brownish blue precipitate (Evans, 1996).

Test for Anthraquinones

❖ Bontrager's test

To a portion of the extract in a dry test tube, 5ml of chloroform was added and shaken for at least 5mins. This was filtered and the filtrate shaken with equal volume of 10% ammonium solution, bright pink colour in the aqueous upper layer indicates the presence of free anthraquinones (Evans, 1996).

Quantitative Phytochemical screening of *I. asarifolia* leaves

Preparation of Fat free Sample

About 2g of the powdered leaf was weighed and defatted with 100ml of diethyl ether using a soxhlet apparatus for 2hours.

- Alkaloid Determination using Haborne (1973) Method
- Flavanoid Determination by the Method of Bohm and Kocipal – Abyazan (1994)
- Saponin Determination
The method of Obadoni and Ochuko (2001) was used.
- Tannin Determination by Van-Burden and Robinson (1981) Method
- Determination of Total Phenols by Spectro-photometric Method measured at 505nm.

Elemental analysis of *I. asarifolia* powdered leaves

The elemental analyses of the plant materials were carried out in Ahmadu Bello University Zaria, Multi-user Research Laboratory. Powdered plant material was digested using 2.5ml of hydrochloric acid (HCl) and 7.5ml Nitric Acid (HNO₃). The concentration of Fe, Mg, Zn, Cu was read using the flame atomic absorption spectrophotometer (FAAS), AA 500 model, Atomic Emission Spectrophotometer. Atomic Absorption Spectrophotometer were used for other elements. Before determining the concentration of any element in the sample, calibration curve of the element in the sample was prepared using prepared standard stock solutions for the elements as reported by AOAC, 2000; 2005; Akpabio and Ikpe (2013).

Acute toxicity studies of methanolic leaf extract of *I. asarifolia*

Lorke method (1983) was adopted for the acute toxicity test in rats. Thirteen (13) of male animal species (*Rattus norvegicus*) were used. Nine (9) rats in 3 groups each of 3 animals per group for the three graded doses of 10,100 and 1000mg/kg were treated orally per body weight and observed for 24hrs for signs of changes in the behavioral pattern and/or death. In a 2nd phase of the experiment, the remaining 4 rats in each of the 4groups of one animal per group respectively were given lower or higher doses of *I. asarifolia* leaves depending on the occurrence of death or no death in the first phase and observed again for 24hrs. The oral median lethal doses were then calculated as geometric mean of the highest non-lethal and the lower lethal doses as follows:

$LD_{50} = \text{Maximum non lethal dose} * \text{Minimum lethal dose for both animal species}$ (Lorke, 1983).

Results

Chemo-microscopical studies on leaves of *I. asarifolia* were found to have cellulose cell wall, lignin, calcium oxalate crystals, tannins, starch and mucilage but calcium carbonate was absent.

Table 1 Chemomicroscopical properties of *I. asarifolia* powdered leaves

Constituents	Inference
Starch	+
Gum and Mucilage	+
Cellulose cell walls	+
Lignin	+
Aleurone grain	+
Calcium oxalate crystals	+
Calcium carbonate	-
Suberized/Cuticular cell wall	+
Inulin	+

+ = Present

- = Absent

Table 2. Physicochemical Constants of *I. asarifolia* powdered leaves

Parameters	Values (%w/w) ± SEM*
Moisture content	5.22±0.11
Ash content	10.89±0.83
Acid insoluble ash	3.17±0.833
Water soluble ash	7.88±0.60
Water extractive value	16.33±0.88
Ethanol extractive value	25.68±0.88

*Average values of three determinations, SEM= Standard error mean

Phytochemicals which include alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates and triterpenes were detected in both aqueous and methanolic extracts while steroid is absent. Anthraquinones were detected in the methanolic extract but absent in aqueous extract of *I. asarifolia*.

Table 3. Qualitative and quantitative phytochemical screening of aqueous and methanolic leaf extract of *I. asarifolia*

Metabolite	Inference		Quantity (mg/g)
	Aqueous	Methanolic extract	
Alkaloid	+	+	130±0.0
Flavonoid	+	+	148±0.0
Saponins	+	+	5.0±0.0
Cardiac glycoside	+	+	-
Tannins	+	+	42.8±0.0
Steroid	-	-	-
Triterpenes	+	+	-
Phenol	+	+	8.0±0.0
Anthraquinones	-	+	-
Carbohydrate	+	+	-

Trace metals which include Fe, Mn and Ni detected in *I. asarifolia* were below the FAO/WHO (1984) permissible limit for edible plants. While others, Pb, Zn, Cd and Cu were found to be within the safety limit.

Table 4: Elemental analysis of *I. asarifolia* powdered leaves

Elements	Concentration (ppm)	FAO/WHO (1984) limit* (ppm)
Iron(Fe)	6.100	20.00
Copper (Cu)	0.200	3.00
Lead (Pb)	0.470	0.43
Zinc (Zn)	2.100	27.40
Nickel (Ni)	0.430	1.63
Manganese (Mn)	3.850	2.00
Aluminum (Al)	7.200	-
Cadmium (Cd)	0.001	0.21
Selenium (Se)	0.410	-
Chromium (Cr)	0.021	-
Arsenic (As)	0.230	-

Table 5. Acute toxicity studies of methanolic leaf extract of *I. asarifolia*

Plant species	Group	Number of Animals	Dose (mg/kg)	Mortality recorded after 24hrs
Phase I	I	3	10	0/3
	II	3	100	0/3
	III	3	1000	0/3
Phase II	I	1	1500	0/1
	II	1	2250	0/1
	III	1	3250	0/1
	IV	1	5000	0/1

Discussion

The primary and secondary metabolites in plants have numerous functions. Crude, pure and isolated alkaloids and their synthetic derivatives have been used as analgesic, antispasmodic and bactericidal agents (Stary, 1998; Okwu and Okwu, 2004). Flavonoids have been shown to provide antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic and vasodilatory activity (Alan and Miller, 1996). Flavonoid also has immense antioxidant and antiinflammatory activity because of its ability to scavenge hydroxyl radicals, super oxide anions and lipid peroxy radicals (Okwu and Josiah, 2006). Tanins have been used in the treatment of wounds especially those emanating from varicose ulcers and hemorrhoids (Njoku and Akumufula, 2007) and is able to stop bleeding during circumcision (Edeoga *et al.*, 2005). The phytochemical constituents especially the secondary metabolites could be useful as guide to chemotaxonomic markers (Jonathan and Tom, 2008) that will aid in chemo taxonomical classification system and further phylogenetic studies in Convolvulaceae family.

The chemo-microscopic features are most valuable in the identification of powdered drug as their identification is largely based on the form, the presence or absence of certain cell types and cell inclusions. These are very important diagnostic pharmacognostic parameters for the identification and authentication of crude drugs especially in powdered plants (Chanda, 2011). Physicochemical parameters evaluated showed the presence of moisture content (5.22%). It is very essential to control moisture content since higher moisture content in plant material may lead to its deterioration and may therefore result in biodegradation of active constituents. Less moisture content is also an indication that the plant material can be kept for some time (Prasad *et al.*, 2013). Total ash value present both physiological and non physiological ash from the plant drugs and non-physiological ash is an indication of inorganic residues after the plant drug is incinerated while acid insoluble ash values of these studies indicates that the plant were in good

physiological condition and it contained little extraneous matters such as sand, silica and soil. A high ash value is indicative of contamination, substitution, adulteration or carelessness in preparing the crude drug for marketing. Acid insoluble ash indicates contamination with silica, for example, earthy materials and sand. Water soluble ash is that part of the total ash content which is soluble in water. It is good indicator of the water soluble salts in the drug. Total ash value content was 10.89%, acid insoluble ash was 3.17% and water soluble ash value was 7.88%. The water and ethanol extractive value was determined to be 25.68% and 16.33% respectively.

Phytochemicals are naturally occurring biologically active, non nutritive chemical compounds found in plants and act as a natural defense system against various pests. Various phytochemicals have been known to possess medicinal properties and hence widely used in Nigerian system of traditional medicine. In this study, various phytochemicals like alkaloids, flavonoids, saponins, phenols, tannins, glycosides, carbohydrates and triterpenes were detected in both aqueous and methanolic extracts while steroid is absent. Anthraquinones were detected in the methanolic extract but absent in aqueous extract of *I. asarifolia* indicating their potential medicinal uses. Phytochemical investigation of the leaf extract of *I. asarifolia* led to the identification of saponins, anthraquinones, phenols, tannin, alkaloid, flavonoids e.g. rutin (Pale *et al.*, 2003; Jegede *et al.*, 2009; Aliyu *et al.*, 2011). Different phytochemicals have been found to possess a wide range of activities which may help in protection against chronic diseases. For example, saponins, flavonoids, tannins and alkaloids have been reported with hypoglycemic and anti-inflammation activities (Argal *et al.*, 2006). The presences of phenolic compounds which are known to have antibacterial activity were revealed in this plant. This therefore, supports the use of the plant in the traditional treatment of cutaneous infections, venereal diseases and dysentery (Thomas *et al.*, 2008). Reports show that saponins possess hypocholesterolemic and antidiabetic properties. The terpenoids have also been shown to decrease blood sugar level in animal studies (Rupasinghe *et al.*, 2003). Trace metals such as Fe (6.10ppm), Mn (3.85ppm) and Ni (0.43ppm) detected in *I. asarifolia* were below the FAO/WHO (1984) permissible limit for edible plants. While others: Pb (0.47ppm), Zn (2.10ppm), Cd (0.001ppm) and Cu (0.20ppm) were found to be within the safety limit.

The elements are rich sources of macro and minor elements that aid in the growth of plants, and as well in human body functions such as muscle contraction, bone formations, growth, metabolism, osmotic balance, regulatory processes activation and other organic bimolecular activities (Rabia *et al.*, 2012). The concentrations of elements gotten from this study were within FAO/WHO (2004) permissible limits for edible plants. Zinc (Zn) is an essential component of a number of enzymes present in animal tissue including alcohol dehydrogenase, carbonicanhydrase, procarboxypeptidase and aids in normal growth, reproduction, tissue repair and wound healing. Zinc deficiency causes growth retardation and skin lesions (Chatterjee and Shinde, 1995). Manganese (Mn) is often found in minerals in combination with iron. It is helpful in carbohydrate metabolism and served in the body as a co-factor for the enzymes involved in hydrolysis, phosphorylation, transamination and decarboxylation. It promotes the activities of transferases such as superoxide dismutase and aids as antioxidant to scavenge damaging particles (superoxide) known as free radicals in the body (Dias, 2012). Low levels of manganese can cause infertility, bone malformation weakness and seizures. The main function of iron is the transport of oxygen to the tissues (haemoglobin) and also in cough associated with angiotensin-converting enzyme (ACE) inhibitors, haemopoietic and cell mediated immunity (Faizul *et al.*, 2012). The deficiency of iron has been related to anemia and

described as the most prevalent nutritional deficiency. The systemic decrease in Copper levels causes iron deficiency. Therefore it is antianaemic and essential for the formation of iron and haemoglobin. Copper (Cu) play important role in treatment of chest wounds and prevent inflammation in arthritis and similar diseases (Faizul *et al.*, 2012).

Besides macro and trace elements, heavy metals were also present in the leaves of *Ipomoea asarifolia* but in negligible concentrations that will not cause harm when consumed or ingested as prescribed by WHO (2007). Though, it is advisable not to be taken for a long period of time to prevent untoward effects. Cobalt (Co) is required in very small amounts in all mammals and is used to treat several different types of cancer in humans and to treat anaemia, but the intake of its high amount can cause heart diseases (Faizul *et al.*, 2012). Lead (Pb) is toxic and a non-essential element for human body, it causes a rise in blood pressure, kidney damage, miscarriages and subtle abortion, brain damage, declined fertility of men through sperm damage, diminished learning abilities of children and disruption of nervous systems (Khan *et al.*, 2011 and Obiajunwa *et al.*, 2002).

The oral median lethal doles (LD₅₀) for the methanolic extract of *I. asarifolia* was estimated to be greater than 5000mg/kg. With careful observations of experimental animals from the first 30 minutes up to the 24hrs, it was revealed that there were no deaths and any sign of toxicity such as loss or increase in weight, tiredness, abdominal constrict convulsion, hyperactive, weakness, diarrhea or increased diuresis within the short and long term effect in rats dosed with 5000 mg/kg b.w of the *I. asarifolia* extracts (methanol). The outcome of the study of Alhassan *et al.*, (2014) gave an LD₅₀ of 2000 mg/kg and this guided our choice of dose used. The LD₅₀ was found to be greater than 5000 mg/kg body weight orally, and this suggested that the extract has low acute toxicity when administered orally.

This may be attributed to the incomplete absorption brought about by inherent factors limiting absorption in the gastro intestinal tract (Dennis, 1984). The present study agrees with the work done by Prasanth *et al.*, (2015); Ugbogu *et al.*, (2016); Kofi *et al.*, (2014) and Adesegun *et al.*, (2016). Bruce, (2006) reported that any substance with LD₅₀ estimated to be greater than 2000 5000 mg/kg body weight given orally could be considered to be of low toxicity and safe.

Similarly, the chemical labelling and classification of acute systemic toxicity based on oral LD₅₀ values recommended by the OECD and (Walum, 1998) are as follows: less than 5 mg/kg: very toxic, greater than 5 but less than 50 mg/kg: toxic, greater than 50 but less than 500 mg/kg: harmful, and, greater than 500 but less than 2000 mg/kg: no label. The very high LD₅₀ observed is not a conclusive finding about the safety of the extracts of *I. asarifolia* as higher doses could be tested for better understanding of its effects if use for a long period of time and for proper recommendation on its future utilization (Olson *et al.*, 2000; Rang *et al.*, 2001; Maikai *et al.*, 2008; Ogonnia *et al.*, 2011).

Conclusion

Ipomoea asarifolia leaves possess secondary metabolites which include alkaloids, tannins, flavonoids, cardiac glycosides and saponins. The values of Fe, Mn and Ni in the plant were below the FAO/WHO (1984) permissible limit for edible plants. However, Pb, Zn, Cd and Cu were found to be within the safety limit. The Acute toxicity (LD₅₀) of the methanolic leaf extract

of *I. asarifolia* was found to be greater than 5000 mg /kg and is considered safe for use. Nonetheless, further studies are encouraged to evaluate toxicity at much higher doses.

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