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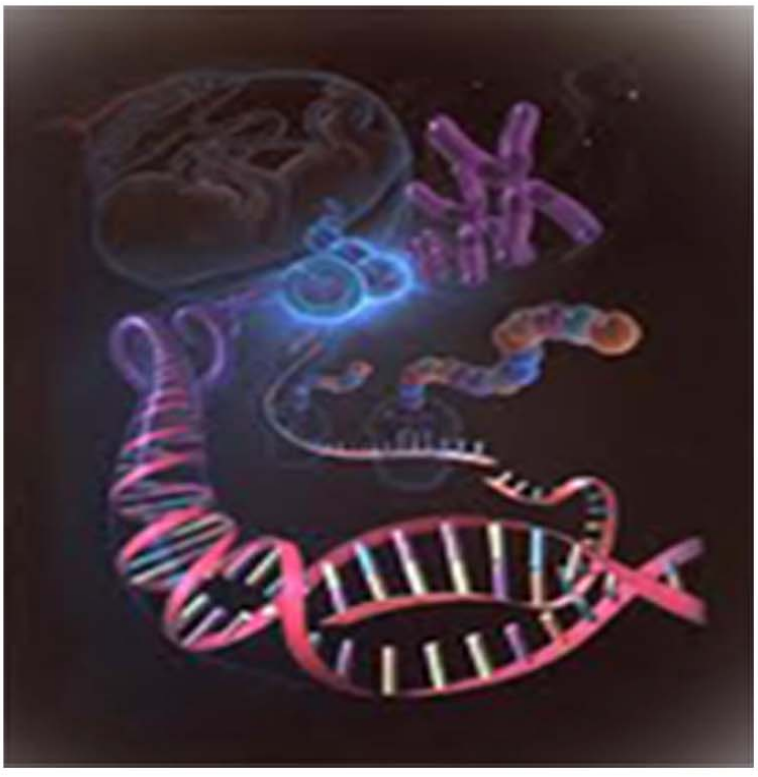

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Research Paper

PHYTOCHEMICAL AND ACUTE TOXICITY STUDIES OF MORINGA OLEIFERA ETHANOL LEAF EXTRACT

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Acute toxicity and phytochemical studies of ethanol leaf extract of *Moringa oleifera* were analysed. The phytochemical analyses of the ethanol extract of *Moringa oleifera* leaf showed the presence of tannins, carbohydrates, saponins, glycosides, reducing sugars, terpenoids, steroids, flavonoids and alkaloids. Phytochemicals such as resins, proteins and fat oils were not detected. The LD_{50} of the ethanol leaf extract in mice was found to be less than 5000mg/kg body weight.

Keywords: *Moringa oleifera*, Phytochemicals, LD_{50}, body weights, Flavonoids and reducing sugars

INTRODUCTION

Phytochemicals are chemical compounds that are naturally found in plant. They are responsible for the colour and organoleptic properties of the plant (Liu, 2004). It is also referred to as those chemicals that may have biological significance but are not established as an essential nutrients in plant (Brow and Arthur, 2001). Phytochemicals could be available as a dietary supplements, but the potential health benefits of phytochemicals are derived from consumption of the whole plant (Rao and Rao, 2007).

In most acute toxicity test, a single dose of a test substance is given to an animal. One measure of the acute toxicity is the lethal dose 50 (LD50) which is the dose of a substance that kills 50 percent of the animals tested (Fleming and Hunt, 2000).

Moringa oleifera is considered to be effective in the treatment of many diseases (Caceres *et al.*, 1991). It is an exceptionally nutritious vegetable tree with a variety of potential uses. The tree itself is rather slender, with dropping branches. It is often cut back annually to one meter or less and allowed to re-grow so that its pods and leaves remain within arm's reach. *Moringa* tree is mainly grown in the semi-arid tropical and sub-tropical areas (Amia, 2013). It grows best in dry sandy soil and can tolerate any other type of soil. It is a fast growing drought-resistant tree that is native to the Southern foothills of Himalayans in Northern India. It is considered as one of the world's most useful tree,

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as almost every part of the plant could be used for food or has some other beneficial properties (Anamika *et al*., 2010). In the tropics, it is used as forage for livestock and in many countries as vegetables that has the potential to improve nutrition, boost food security, foster rural development and support sustainable land care.

The immature green pods called drumsticks are probably the most valued and widely used part of the tree. They are commonly consumed in India and are generally prepared in a similar fashion to green beans and have a slight asparagus taste (Foidl *et al*., 2001). The seeds are sometimes removed from more matured pods and eaten like peas or roasted like nuts (Joshua and Vasu, 2013). The flowesr are edible when cooked and are said to taste like mushrooms. The roots are shredded and used as condiments in the same way as *horse radish.*The leaves are highly nutritious, and also considered as source of beta-carotene, vitamin C, protein, iron and potassium (Makkar and Becker, 2007). The leaves are cooked and used like Spinach. In addition to being used as a substitute for Spinach, its leaves are commonly dried and crushed into powder and used in soups and sauces. In Siddha medicine, these drumstick seeds are used as a sexual virility drug for treating erectile dysfunction in men and also in women for prolonging sexual activity.

Moringa oleifera, grown and used in many countries around the world serves as a multi-purpose tree with an established medicinal, nutritious and social-economical values (Bodeker and Willcox, 2000 ; Shivashankarm and Garvit, 2012). In Senegal and Benin, *Moringa oleifera* leaves are dispersed as powder at health facilities to treat malnutrition in children (Willcox *et al*., 2005). It was massively grown and promoted by the local media in Uganda in the 1980s as a plant putatively able to cure a number of diseases including malaria and symptoms of HIV/AIDS (Willcox, 1999).

This study was designed to determine the phytochemicals and acute toxicity of ethanol leaf extract of *Moringa oleifera.*

MATERIALS AND METHODS

Plant Material

Fresh leaves of *Moringa oleifera* were obtained from Ovoko, Igbo-Eze South L G A of Enugu State, Nigeria. The leaves were identified by Mr O Chijioke of the *Hebarium* unit of the Department of Botany, University of Nigeria, Nsukka.

Animals

The experimental animals used for this study were white albino mice of either sex weighing 20-34 g.The mice were between 3-4 months old and were obtained from the animal unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Chemicals/Reagents

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstand, Germany.

Extraction Procedure

The fresh leaves of *Moringa oleifera* plant were plucked and dried under room temperature at ($29^{O}C$-$35^{O}C$) for three weeks, after which the leaves were pulverized into coarse form with a crestor high speed milling machine. The coarse form (130 g) was then macerated in absolute ethanol. This was left to stand for 48 h. After that the extract was filtered through muslin cloth on a plug of glass wool in a glass column. The resulting ethanol extract was concentrated and evaporated

to dryness using rotary evaporator at an optimum temperature was between 40 and 45^{O}C to avoid denaturation of the active ingredients. The concentrated extract was stored in the refrigerator.

Determination of Yeld of Extract

The percentage yield of the extract was determined by weighing the coarse *Moringa oleifera* leaf before extraction and the *Moringa oleifera* ethanol leaf extract after concentration and then calculated using the formula.

$$\text{Percentage}(\%)\text{ yield} = \frac{\text{Weight (g) of the concentrated extract}}{\text{Weight (g) of the ground Moringa leaves}} \times 100$$

Determination of Phytochemicals

The qualitative phytochemical analyses were carried out according to the methods of Harborne (1973) and Trease and Evans (1989).

Determination of Lethal Dose 50 (LD_{50})

Acute toxicity studies (LD_{50}) was measured using method of Lorke (1989). The animals were divided into two groups A and B, with each group sub-divided into four groups made up of three animals each.

RESULTS

Percentage Yield of the Extract

From the result in Table 1 the percentage (%) yield of the ethanol leaf extract of *Moringa oleifera*

Table 1: The Percentage Yield of the Ethanol Leaf Extract of *Moringa oleifera*

Initial Weight of Ground Extract (g)	Final Weight of Extract (g)	Percentage (%) Yield of Extract
130	23.20	17.85

was found to be 17.85%.

Phytochemical Constituents of *Moringa Oleifera* Ethanol Leaf Extract

Phytochemical analyses of the ethanol leaf extract of *Moringa oleifera* showed the presence of tannins, carbohydrates, saponins, glycosides, reducing sugars, terpenoids, steroids, flavonoids and alkaloids. Phytochemicals such as resins, proteins, fats and oil were not detected during the test. The results presented in Table 2 below shows that flavonoids were more in quantity than other phytochemicals detected. Phytochemicals such as carbohydrates, reducing sugars, steroids and alkaloids were moderate in concentration while phytochemicals such as tannins, saponins, glycosides and terpenoids were found to be relatively low in concentration.

Table 2: Phytochemical Constituents of *Moringa oleifera*

Constituents	Ethanol Extract
Tannins	+
Carbohydrates	++
Saponins	+
Glycosides	+
Reducing Sugars	++
Terpenoids	+
Steroids	++
Flavonoids	+++
Alkaloids	++
Resins	ND
Proteins	ND
Fats and Oil	ND

Note: +++ = Relative Abundance of Compound; ++ = Moderate Abundance of Compound; + = Relative low Presence of Compound; ND = Not Detected.

Acute Toxicity (LD_{50})

The LD_{50} of the ethanol leaf extract of *Moringa oleifera* in mice was found to be more than 2600mg/kg and less than 5000 mg/kg body weight. One animal died and the other remaining two animals

Table 3: Acute LD_{50} of the Extract on Mice

Group	Dosage	Mice 1	Mice 2	Mice 3
Phase 1				
Group 1	10 mg/kg	ND and NST	ND and NST	ND and NST
Group 2	100 mg/kg	ND and NST	ND and NST	ND and NST
Group 3	1000 mg/kg	ND and NST	ND and NST	ND and NST
Phase 2				
Group 1	1,900 mg/kg	ND and NST	ND and NST	ND and NST
Group 2	2,600 mg/kg	ND and NST	ND and NST	ND and NST
Group 3	5,000 mg/kg	ST	D	ST
Group 4	Standard Control	ND and NST	ND and NST	ND and NST

Note: ND = No Death; NST = No Signs of Toxicity; D = Death; ST = Signs of Toxicity.

in the group showed signs of toxicity as illustrated below within 24 h of constant observation.

DISCUSSION

Phytochemicals have been used as drugs for Millenia. Hippocrates prescribed willow tree leaves to abate fever. Salicin, an anti-inflammatory agent was originally extracted from the bark of white willow tree and later synthetically produced as the staple over-the counter drug aspirin (Krishnadev *et al*., 2010). Phytochemicals in fruits and vegetables may reduce the risk of cancer possibly due to there dietary fibers, polyphenol antioxidants and anti-inflammatory effects (Brow and Arthur, 2001).

Some phytochemicals with physiological properties may be elements rather than complex organic molecules found in many fruits and vegetables. Selenium is involved with major metabolic pathways that include both the immune function and thyroid hormone metabolism (Brow and Arthur, 2001). There are currently, many phytochemicals in clinical trials for a variety of diseases. Lycopene from tomatoes has been tested in human studies for cardiovascular diseases and prostate cancer (Liu, 2004).

The LD_{50} of the ethanol leaf extract of *Moringa oleifera* in mice was found to be more than 2600 mg/kg and less than 5000 mg/kg body weight.

The results of the phytochemical analyses showed that flavonoids were more in quantity than the other phytochemicals tested. Flavonoids, according to the research by (Mobh, 1938) may modify allergens, viruses and carcinogens thereby acting like a biological response modifier. Also*, in vitro* studies showed that flavonoids could also posses anti-microbial (Galeotti *et al*., 2008), anti-allergic (Spencer, 2008) and anti-inflammatory properties (Yamamato and Gaynor, 2000).

Phytochemicals such as carbohydrates, reducing sugars, steroids and alkaloids were found to be moderate in concentration. Steroids are used in the stimulation of bone marrow and growth. It stimulates lean body mass and also play vital roles in the prevention of bone loss in elderly men (De-piccolli *et al*., 1991).

Alkaloids have pharmacological effects and are used as local anesthetic and stimulants. Cocaine, caffeine, nicotine, the analgesic morphine , the anti-bacterial berberine and anti-malarial drug quinine are all Alkaloids (Manske, 1965).

The results of phytochemicals such as tannins, saponins, glycosides and terpenoids were found to be relatively low in concentration . Tannins could be an effective ameliorative agent of the kidney (Bajaj, 1998). Tannins have also shown to be a potential anti-viral , anti-bacterial and anti-parasitic agents (Lu *et al*., 2004). Saponins are used as an adjuvants in the production of vaccines (Asl and Hossein, 2008).

CONCLUSION

In conclusion, the results of the phytochemicals and acute toxicity shown above have indicated that ethanol leaf extract of *Moringa oleifera* are rich in phytochemicals and at the same time safe for consumption as shown in its lethal dose LD_{50} results. This could be the reason ethanol leaf extract of *Moringa oleifera* has been used in numerous ethnomedicinal practises.

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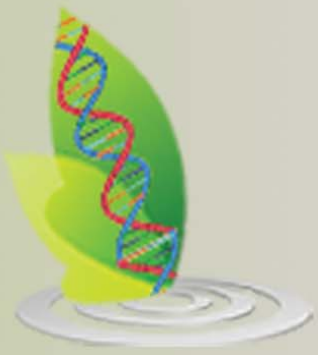

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