International Journal of Phytomedicine 6 (2014) 243-247
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Phytochemical screening and acute toxicity study of *Cucumis metuliferus* E. Mey. Ex. Naudin fruit extract in Cockerels

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Abstract

The ripe fruits of *Cucumis metuliferus* were collected at Vom, Jos South Local Government Area of Plateau State, cleaned, sliced, air-dried, pulverised and cold extracted with solvents of different polarities (n-hexane, chloroform, methanol and distilled water). The ground powder and the extracts obtained were phytochemically screened for their chemical composition. These revealed the presence of saponins, alkaloids, carbohydrates, flavonoids, tannins, cardiac glycosides, steroids and terpenoids. The median lethal dose (LD_{50}) of the methanol extract was determined in chicken by oral (p.o) and intra-peritoneal (i.p) routes. An LD_{50} above 5000 mg/kg body weight was established for both routes, indicating that probably the fruit of C. metuliferus is not toxic.

Keywords: Phytochemical screening, *Cucumis metuliferus*, acute toxicity, LD_{50}, cockerels

Introduction

Various plants have been screened for their phytochemical constitutents [1, 2, 3, 4] and the activity of a plant has been attributed to the phytochemicals present [5]. The plant *Cucumis metuliferus* (Cucurbitaceae) is a monoecious annual herb with staminate flowers that grows wild [6]. It flowers and fruits from July to September and the fruits ripen from October to December [7]. *C. metuliferus* has been shown to cure diseases such as peptic ulcer [8, 9], diabetes [10], Newcastle disease [11]. It has been reported that the seeds and fruits of the plant are eaten raw as food supplement and that it is highly valued for its antihelmintic properties [12]. The fruit pulp has been reported to increase sperm/semen integrity (6). It has been reported that the crude powder of *C. metuliferus* contains alkaloids, saponins, tannins, flavonoids, steroids, cardiac glycosides and carbohydrates but anthraquinones are absent while the ethanol extract contains alkaloids, tannins, flavonoids, steroids, cardiac glycosides and carbohydrates but saponins and anthraquinones are not present [13]. Although the LD_{50} of the plant in rats is shown to be above 5000 mg/kg (14) there is no work on the LD_{50} of *C. metuliferus* in chickens therefore, this work is designed to phytochemically screen the plant and carry out the LD_{50} of the plant in cockerels.

Materials and Methods

Plant Collection and Identification

The ripe fruits of *Cucumis metuliferus* E. Mey. Ex Naudin were collected in November 2012 at Vom, located in Plateau State, Nigeria. The plant was identified and authenticated by a plant taxonomist, Prof. S.S. Sanusi of the Department of Biological Sciences, University of Maiduguri, Maiduguri, Borno State, Nigeria to be *C. metuliferus*.

Preparation and Extraction of Plant Material

The ripe fruits of *C. metuliferus* were cleaned, sliced, air dried and pulverised in the laboratory at the Department of Veterinary Physiology, Pharmacology and Biochemistry University of Maiduguri, Maiduguri, Borno State and this was kept in an air-tight container until used. 1500 g of the pulverised fruit was successively extracted with solvents of different polarities (n-hexane, chloroform, methanol and water) after maceration for 24 h and then filtered. The yield of each extract was calculated.

Phytochemical Screening

The dried ground material and the four extracts were phytochemically screened for the presence of chemical constituents using standard procedures of analysis as described by [15] and Evans [16].

Acute Toxicity Test (LD_{50})

Twenty four (24) cockerels obtained from Ghamba Consultancy and Enterprises, Wulari, Maiduguri, Borno State were kept intensively for six weeks at the Veterinary Physiology and Pharmacology Laboratory. The birds were fed *ad libitum* with Vital

Feed (Vital Feeds Plc, Jos, Nigeria). Water was also given *ad libitum*. The LD_{50} was determined according to Lorke's method [17] [orally and intra-peritoneally].

The LD_{50} was carried out in two phases. In phase I, eighteen birds were grouped into six groups of 3 birds each (Groups A to C for oral administration and groups D to F for intra-peritoneal administration) were dosed with 10, 100 and 1000 mg/kg body weight of the extract at a concentration of 200 mg/ml. They were kept and monitored for mortality for 24 h. In phase II of the study, six birds were grouped into 6 of 1 bird each (Groups A to C for oral administration and groups D to F for intra-peritoneal administration). They were dosed with 1600, 2900 and 5000 mg/kg body weight of the extract at a concentration of 200 mg/ml. After 24 h, mortality was also noted. The median lethal dose (LD_{50}) was calculated as the geometric mean of the least dose that kills a bird and the highest dose that does not kill any cockerel.

$LD_{50} = \sqrt{a \times b}$

Where a = least dose that kills a cockerel

b= highest dose that does not kill any cockerel.

Results

Extraction of Plant Material

The yield and texture of each extract are shown in Table 1.

Table 1: The yield and texture of crude extracts of the fruit of *Cucumis metuliferus*.

S/N	Extract	Yield (%)	Texture of Extract
1	CHE	3.75	oily
2	CCE	3.16	oily
3	CME	7.11	gel-like
4	CAE	16.35	gum-like

Key:
CHE = Crude n-Hexane Extract
CCE = Crude Chloroform Extract
CME = Crude Methanol Extract
CAE = Crude Aqueous extract

Phytochemical Screening

The phytochemical screening of the ground powder and the four extracts (Table 2) showed the various chemical constituents of the different extracts present in the fruit of *C. metuliferus*. The result of the phytochemical screening revealed the presence of useful chemical compounds such as cardiac glycosides, steroid and terpenoids in all the extracts, however, other phytochemicals like alkaloids, carbohydrates, flavonoids,

Table 2: Phytochemical screening of the powdered and the crude extracts of *Cucumis metuliferus* Fruit

Phytochemical Constituent	Type of Test	Inference				
		Ground powder	CHE	CCE	CME	CAE
Alkaloids	Dragendoff's	+	-	-	+	-
	Mayer's	+	-	-	+	-
Anthraquinones	Free Anthraquinone (Borntragers)	-	-	-	-	-
	Combined Anthraquinone	-	-	-	-	-
Carbohydrates	Molisch's (General test)	-	-	-	-	-
	Barfoed's (Monosaccharides)	-	-	-	-	-
	Free reducing sugar	+	-	-	+	+
	Pentoses	-	-	-	-	-
	Ketoses (Salivanoffs)	+	-	-	+	-
Cardiac glycosides	Salkowski's (for steroid ring)	+	+	+	+	+
	Lieberman-Burchard's	+	+	+	+	-
Flavonoids	Shinoda's	-	-	-	+	-
	Ferric chloride	-	-	-	-	-
	Lead acetate	+	-	-	+	+
	Sodium hydroxide	-	-	-	-	-
Phlobatanins	Hydrochloric acid	-	-	-	-	-
Saponins	Frothing	+	-	-	+	+
Soluble starch		-	-	-	-	-
Steroids		+	+	+	+	+
Tannins	Ferric chloride	-	-	-	+	-
	Lead acetate	+	-	-		+
Terpenoids		+	+	+	+	+

Key:
– = Absent + = Present

saponins and tannins were present in the ground powdered fruit, methanol and water extracts but are absent in n-hexane and chloroform extracts. Soluble starch, phlobatannins, free and combined anthraquinones are absent in all extracts.

Acute Toxicity Test (LD_{50})

There was no mortality of birds recorded after the administration of the methanol extract for the two phases, therefore the LD_{50} of the plant *Cucumis metuliferus* is above 5000 mg/kg body weight (Table 3) for both oral and intra-peritoneal routes.

Table 3: Acute toxicity test of the crude methanol extract of *C. metuliferus* administered in cockerels orally (p.o) and intra-peritoneally (i.p)

Phase	Group	No. of Birds	Route	Dose (mg/kg bd. Wt.)	No. of Death
1	A	3	Oral	10	0/3
1	B	3	Oral	100	0/3
1	C	3	Oral	1000	0/3
1	D	3	Intraperitoneal	10	0/3
1	E	3	Intraperitoneal	100	0/3
1	F	3	Intraperitoneal	1000	0/3
2	A	1	Oral	1600	0/1
2	B	1	Oral	2900	0/1
2	C	1	Oral	5000	0/1
2	D	1	Intraperitoneal	1600	0/1
2	E	1	Intraperitoneal	2900	0/1
2	F	1	Intraperitoneal	5000	0/1

Since there was no death recorded at the highest dose, the LD_{50} was considered to be more than 5000 mg/kg body weight in both p.o and i.p routes.

Discussion

The plant *C. metuliferus* has several groups of secondary metabolites which account for its use as food or in the treatment of various ailments. The result revealed the presence of useful secondary metabolites such as alkaloids, carbohydrates, cardiac glycosides, flavonoids, saponins, tannins, steroids and terpenoids. This result is in agreement with the work of [10, 13], eventhough, (10) reported the absence of tannins in the fruit of *C. metuliferus*. Reducing sugars have hypoglycaemic effects [18]. Cardiac glycosides known to have cathartic and laxative effects are also used in the treatment of congestive heart failure, constipation, oedema and microbial infections [19, 20]. The glycoside fraction extracted from the fruit pulp of *C. metuliferus* had anti hyperglycaemic activity [10]. In dogs and cats, cardiac glycosides are indicated for their negative chronotropic effect in supraventricular arrhythmias such as atrial fibrillation. They slow the rate of impulse conduction through the atrioventricular node and allow the ventricular rate to fall below the atria and so restore more efficient pumping [21]. So, the cardiac glycosides from the methanolic extract of *C. metuliferus* may probably be used in arrhythmias. Tannins are diverse organic substances with various compositions that have pronounced physiological astringent properties that hasten the healing of wounds and inflamed mucous membrane [22]. Tannins also decrease bacterial cell proliferation by blocking key enzymes of microbial metabolism [23]. Flavonoids have been referred to as nature's biological response modifiers because of their ability to modify the body's reaction to allergies, viruses and carcinogens; they show antimicrobial activity [24]. So the antisalmonella effect demonstrated by this extract may beconnected to this. Flavonoids extracted from *C. metuliferus* have been shown to have antiviral properties [25]. Tannins and flavonoids have been shown to inhibit the growth of *Staphylococcus epidermidis*, *Streptococcus viridans* and *Escherichia coli* [26]. Flavonoids, tannins and saponins were also reported to have inhibitory effect on the growth of *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* [27, 28]. However, some research indicated that only a small amount of flavonoids are necessary to notice its medical benefits. Taking large dietary supplements provides no extra benefit and may pose some risks [29]. So because of the reported flavonoids in the fruit of *C. metuliferus*, it may be used as a neutraceutical. Alkaloids have varied medicinal properties which include anti-protozoal [30)]anticancer, anti inflammatory, analgesic and central nervous system effects [31]. Conine, an alkaloid has been reported to be effective in preventing blood lose during cuts and also bring about blood clot [32]. Alkaloids extracted from the fruit of *C. metuliferus* were shown to have anti-ulcer [9] and anti viral [11] properties.

In acute toxicity testing (Table 3), the median lethal dose (LD_{50}) above 5000 mg/kg body weight of the extract for both routes (p.o and i.p) in cockerels indicates that the plant *C. metuliferus* is safe and non toxic for all practical purpose. According to Clarke and Clarke [33], substances with oral LD_{50} of 5000 to 15,000 mg/kg are slightly toxic and could be administered with some degree of safety, especially through the oral route. The methanol extract also had an i.p LD_{50} of >5000 mg/kg body weight. Dry substances whose i.p LD_{50} fall between 50 and 500 mg/kg are regarded as toxic, between 500 mg/kg but less than 1000 mg/kg are moderately toxic and greater than 1000 mg/kg are not toxic [33, 34]. Therefore, the i.p LD_{50} of *C. metuliferus* fruit of >5000 mg/kg may be regarded as non toxic.

Conclusion

In conclusion, this study revealed the various phytochemical constituents of the ground powder and the different extracts of the fruits of *C. metuliferus.* An LD_{50} above 5000 mg/kg (p.o and i.p) in chickens proved its probable safety, thus, the use of *C. metuliferus* plant in traditional medicine and as food (nutraceutical) may therefore be justified.

Conflict of Interest

Authors have declared that no conflicting interests exist.

Author's Contribution

This work was carried out in collaboration between all authors. UJG designed the study, wrote the protocol and wrote the first draft of the manuscript. UJG and OAS performed the phytochemical analyses and literature searches of the study. UJG and SUK performed the acute toxicity study. All authors read and approved the final manuscript.

Acknowledgements

I acknowledge the technical and experimental support of Phar. J. Gotep of National Veterinary Research Institute (NVRI) Vom, Plateau State and Mr. F. Akanwu of Chemistry Department, University of Maiduguri, Borno State, Nigeria.

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