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Phytochemicals screening and Acute Oral Toxicity Study of *Cucumis metuliferus* fruit extract in Wistar Albino rats

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Abstract:
Aim*: The aim of the study was to determine the phytochemicals profile and acute oral toxicity of Cucumis metuliferus fruit extract*
Materials and Methods*: The non-bitter form of Cucumis metuliferus fruits were harvested with the help of a taxonomist from a farm in Wamumu ward, Mwea west Sub-County, Kirinyaga County in Kenya. Ethanol extraction was done through maceration technique. Standard phytochemical screening procedures were used to test for the fruit's phytochemicals. Twelve (12) nulliparous and non-pregnant Wistar Albino rats weighing between 170 to 190 grams and aged 8 weeks were used for toxicity study as per OECD test guideline number 423.*
Results*: Cucumis metuliferus fruit extract (CMFE) contained high quantities of glycosides, moderate quantities of Alkaloids and low quantities of both Saponins and Tannins. Flavonoids, sterols and steroids were not present. Dosing of Cucumis metuliferus fruit extract at 50mg/kg, 300mg/kg and 2000mg/kg produced no signs or symptoms of acute Oral toxicity. However, when treatment groups were compared with control group at doses of 300mg/kg and 2000mg/kg of Cucumis metuliferus fruit extract, it was noted that there was increased physical activity and change in behaviour pattern (jumping, increased movement within the cage, making noise and increased eating habit). There was no significant difference (P=0.723) when mean body weight was compared between control group and treatment groups.*
Conclusion: *Phytochemical screening confirmed presence of glycosides, Alkaloids, Saponins and tannins in CMFE. There were no toxicity signs and symptoms observed at doses of 50mg/kg, 300mg/kg and 2000mg/kg of CMFE concluding that the possible oral toxic dose is likely to be higher than 2000mg/kg body weight. However, there was remarkable increase in physical activities and appetite to food at doses of 300mg/kg and 2000mg/kg of CMFE implicating that the fruit could be used as an appetizer.*
Key Word*: Cucumis metuliferus; Analgesic; Antimalarial; Antidiabetic; Antitumor; Diuretic; Taxonomist; Phytochemicals; Maceration technique.*

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I. Introduction

In present times, researchers in pharmacology are increasingly studying medicinal plants across the globe and according to World Health Organization (WHO), more than 80% of people across the globe do not seek conventional medicine for their primary health care services rather they rely on traditional medicine where majority use plants or their products (Gupta et al, 2005)). Reports of various plants used in the treatment of diseases like diarrhea, tuberculosis, diabetes mellitus and skin disease have been documented in countries like: Nigeria (Sofowora, 2008); Kenya (Matu & van Staden, 2003); Ethiopia (Gedif & Hahn, 2003); Turkey, Italy, Panama, among others. These herbal options may be the only alternative in places where modern medical care services are not available or affordable. Treatment failure due to microbial resistance to commercial drugs has also necessitated the search for new anti-microbial substances from other sources like plants. Cucumis Metuliferus and other plants of the family Cucurbitaceae have been documented as some of the plants of medical value (Usman, Sodipo, Kwaghe & Sandabe, 2015).

The fruit is native to Central and South Africa although it is now grown all over the world (Dhale, 2011). Accordingly, Dhale (2011) documented that phytochemicals present in Cucumis metuliferus include, saponins, tannins, alkaloids and glycosides. These phytochemicals are effective against some illnesses caused by virus, fungi,

and bacteria as well as having analgesic, antimalarial, antidiabetic, antitumor and diuretic activity (Kwaghe et al., 2015). However, scientific data to support the fruit's safety profile is scanty

II. Material And Methods

Study Area: The study was conducted in the Small Animal Facility for Research and Innovation (SAFARI) at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Kenya between December 2019 and April 2020.

Study Design: This was a Laboratory based experimental study design.

Plant collection: The non-bitter form of *Cucumis metuliferus* fruits were collected from a farm in Wamumu ward, Mwea west Sub-County, Kirinyaga County, Kenya with the help of a taxonomist and taken to botanical department of Jomo Kenyatta University of Agriculture and Technology (JKUAT) for identification and confirmation. A voucher specimen (Voucher NO: DMM-JKUATBH 001A-2019) was deposited in the JKUAT botany herbarium for future reference.

Fruit Extraction procedure: Maceration technique was used to extract the fruit. Whole fruits were washed off dirt and any foreign bodies using clean water then air dried. The fruits were then cut into two equal halves, the content was scooped using a wooden spatula, put in a collection jar and then soaked in ethanol containing air tight glass vessel. At room temperature, the mixture was allowed to stand while shaking occasionally in an orbital shaker for 48 hours. The liquid was strained off and the solid residue pressed to recover as much as occluded solution. The solution was then filtered off particles using muslin cloth followed by filter paper. Ethanol was then evaporated using rotary evaporator and freeze drying followed to remain with *Cucumis metuliferus* fruit extract paste. The paste was stored at 4 degrees Celsius until use.

Animal acquisition and handling: Twelve (12) nulliparous and non-pregnant Wistar Albino rats weighing between 170 to 190 grams and aged 8 weeks were procured and kept in appropriate rat's cages in SAFARI animal house, JKUAT. The rats were treated to 12 hours light/darkness cycle and they had free access to approved rodents pellet food from Unga Feeds Limited and clean water. They were handled humanely and the rules and regulations of SAFARI animal house were observed at all times.

Methodology:

Phytochemical screening procedure

Test for glycosides: 1 ml of extract was mixed with 1 ml of glacial acetic acid and then treated with one drop of 5% ethanolic chloride solution. 1 ml of concentrated sulphuric acid was carefully poured down the side of the test tube. The appearance of a brownish ring between the two formed layers with the lower acidic layer turning blue green upon standing would indicate the presence of cardiac glycosides.

Alkaloids test: 1 ml of the extract was tested with Mayer's reagent prepared by dissolving 35g of mercury chloride in distilled water and a solution of 5grams potassium iodide into 10 ml of water. The mixture was diluted to 100ml. the appearance of opalescence or yellow precipitate would indicate presence of alkaloids.

Flavonoids test: 1ml of the extract was put in to a test tube followed by addition of hydrochloric acid (4 drops) and magnesium turnings. Development of a pink or magenta red would indicate presence of flavonoids.

Sterols and steroid test: 1ml of the extract was put into a test tube in which 0.5ml sulphuric acid, acetic anhydride and chloroform in similar amounts was added. A red coloration would indicate presence of sterols. A green colour would indicate presence of steroids.

Saponins test: 1ml of the extract was put in a test tube then 50 ml of tap water was added. The mixture was shaken vigorously and if persisting honeycombs formed, this would be subjected to confirmatory tests. This involved dissolving 1ml of the extract in anhydride tetrachloride to which 4 drops of concentrated sulphuric acid was added to the mixture. A blue, green or red colour accompanied by a pink ring would show presence of Saponins.

Tannins test: I ml of the extract was dissolved in water in which 1% gelatin salt reagent containing 1% gelatin and 10% sodium chloride and a salt solution (10% NaCl) was added. Presence of tannins would be indicated by presence of blackish blue colour while catechol tannins, greenish black colour.

Acute Oral Toxicity Study

Testing Procedure: This was conducted using up and Down procedure as described by Organization for Economic Co-operation and Development (2001) test guideline number 423. Twelve (12) nulliparous and non-pregnant Wistar Albino rats weighing between 170 to 190 grams and aged 8 weeks were divided into four (4) groups of 3 rats in each. Group one served as the control and was given distilled water. Group 2, 3 and 4 were treatment groups and were given CMFE at doses of 50mg/kg, 300mg/kg and 2000mg/kg respectively. Starting dose of 50mg/kg was selected from one of the four fixed levels, 5, 50, 300, and 2000 mg/kg according to OECD test guideline number 423. Prior to dosing, rats were fasted overnight for 6-8 hours, weighed and after dosing, food was withheld for 4 hours. However, they had free access to drinking water during fasting period. Calculated dose of freeze dried

CMFE was formulated to an aqueous solution by adding distilled water to make a concentration of 1 ml distilled water: 200 mg CMFE. Dosing was done at intervals of 48 hours from one step to the other.
Observation for signs and symptoms was made by paying special attention to skin and fur changes, eyes and mucous membrane changes, changes in physical activity and behaviour pattern changes (jumping, movement within the cage, making noise and eating behaviour) and change in weight. The rats were also observed for, diarrhoea, salivation, tremors, convulsions, lethargy, sleep, coma and death.

Statistical analysis

Raw data was keyed in into Microsoft Excel Spreadsheet and then transferred to Statistical Package for Social Sciences (SPSS) software for analysis. Descriptive statistics such as mean, median, frequencies and standard deviation were generated while clinical data was analysed through description. Comparison of multiple means was done using ANOVA. Tukey statistical test was used for Post hoc analysis. Analysis was done at 95% level of confidence ($P<0.05$). Data was presented in tables

Ethical consideration

Ethical approval was sought from JKUAT Animal Ethics Committee. Ethical conduct was observed at all times during the entire period of the study.

III. Result

***Cucumis metuliferus* fruit extract yield**

The ripe fruit had a percentage yield of 1.15 following ethanol extraction through maceration technique (Table 1).

Table 1: Percentage yield of *Cucumis metuliferus* fruit ethanolic extract

Plant	Weight of the fruit extract (Raw)	Weight of freeze-dried extract (Paste)	Percentage yield
Cucumis metuliferus fruit (Ripe)	3200 grams	36.7 grams	1.15

Phytochemicals present in *Cucumis metuliferus* fruit extract

Phytochemical screening revealed that *Cucumis metuliferus* fruit extract contained high quantities of glycosides, moderate quantities of Alkaloids and low quantities of both Saponins and Tannins. Flavonoids, sterols and steroids were not present.

Table 2: Phytochemicals present in *Cucumis metuliferus* ethanolic fruit extract

Phytochemical	Quantity
Glycosides	+++
Alkaloids	++
Flavonoids	-
Sterols and Steroids	-
Saponins	+
Tannins	+

Acute oral toxicity study of CMFE results

Dosing at 50mg/kg produced no signs or symptoms of acute Oral toxicity. This was also observed at doses of 300mg/kg and 2000mg/kg. The upward dose progression factor was employed to reach a dose limit of 2000mg/kg because none of the lower doses produced mortality. There were no changes observed for skin and fur, eyes and mucous membrane. However, there was noted increase in physical activity and change in behaviour pattern (jumping, movement within the cage, making noise and eating behaviour). The rats did not present with diarrhoea, salivation, tremors, convulsions, lethargy, sleep and coma (Table 3).

There was no significant difference ($P=0.723$) when mean body weights were compared between control group and various toxicity test groups (Table 4.4)

Table 3: Acute oral toxicity observation schedule results

Group	Treatment	Route of administration	Observations schedule						Mortality
			0 Hr	½ Hr	1 Hr	24Hrs	48Hrs	Daily for 14 days	
1	Distilled water	Oral gavage	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3
2	CMFE 50(Mg/Kg)	Oral gavage	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	Normal activity	0/3
3	CMFE 300(Mg/Kg)	Oral gavage	Normal activity	Normal activity	Increased physical activity and behaviour	Increased physical activity and behaviour	Increased physical activity and behaviour	Normal activity	0/3
4	CMFE 2,000(Mg/Kg)	Oral gavage	Normal activity	Normal activity	Increased physical activity and behaviour	Increased physical activity and behaviour	Increased physical activity and behaviour	Normal activity	0/3

Table 4: Comparison of mean body weight between various toxicity test groups

Groups	N	Mean	Std. Deviation
Group 1 (Control)	12	202.37	19.15883
Group 2 (50mg/kg CMFE)	12	207.53	16.96758
Group 3 (300mg/Kg CMFE)	12	204.31	17.83186
Group 4 (2000mg/Kg CMFE)	12	199.18	18.72542

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	440.961	3	146.987	.444	.723
Within Groups	14559.339	44	330.894		
Total	15000.300	47			

IV. Discussion

Phytochemical screening of *Cucumis metuliferus* fruit extract revealed presence of high quantities of glycosides, moderate quantities of Alkaloids and low quantities of both saponins and Tannins. These study findings agreed with a study conducted by Dhale (2011). Another study conducted by Maluleke et al. (2021) confirmed that Cucumis metuliferus fruit grown in Florida science campus in South Africa contained Flavonoids and phenols. A study conducted by Ezekaibeya et al. (2020) confirmed presence of glycosides, flavonoids, alkaloids, saponins, tannins, steroids, terpenoids, phenols in *Cucumis metuliferus* fruit rid collected in Nigeria.

At a limit dose of 2000mg/kg body, CMFE exhibited no toxicity suggesting that LD50 could be more than 2000mg/kg body weight. This is in line with a study conducted by Usman, J.G. & Sodipo, Olufunke & Sandabe, Umar. (2014) which showed that at limit dose of 5000mg/kg body weight, CMFE produced no toxicity signs and symptoms when administered orally to cockerels. This suggests that the extract's safety profile at doses of 2000mg/kg is relatively safe. However, it was noted that at 300mg/kg and 2000 mg/kg body weight, physical activity (jumping, movement within the cage, making noise and eating habit) was increased. The findings agreed with Jimam et al. (2012) who demonstrated that there was increased appetite in Wistar rats when *Cucumis metuliferus* fruit extract was administered orally to the rats.

V. Conclusion

The study confirmed presence of glycosides, Alkaloids, Saponins and tannins which could be implicated in the increased appetite observed at doses of 300 mg/kg and 2,000 mg/kg body weight of CMFE. Having no evidence of toxicity when CMFE was administered orally at doses of 50mg/kg, 300mg/kg and 2000mg/kg body

weight, this study suggested that LD_{50} is likely to be higher than the highest dose administered i.e. a dose of 2000mg/kg body weight.

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