Cytotoxicity of *Citrullus lanatus* (Thunb) Mansf. on *Artemia salina* (Brine Shrimp Test)

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ABSTRACT

A study on cytotoxicity of Citrullus lanatus (water melon) extracts on Artemia salina (Brine shrimp test (BST) was carried out using extraction and cytotoxicity standard procedures, to determine cytotoxic effects of the plant extracts . The stems, leaves and seeds of light bark variety of C. lanatus were collected from karfi plantation site, Kano State, Nigeria. The plant parts were air dried, pulverized and extracted using 95% ethanol. The three ethanolic extracts from stems, leaves and seeds were further fractionated using chloroform, water, 90% methanol and petroleum ether. The three extracts and twelve fractions namely: ethanol soluble extracts, chloroform soluble fractions, water soluble fractions, methanol soluble fractions and petroleum ether soluble fractions from stem leaves and seeds were subjected to the brine shrimp test. The ethanol extracts and water fraction of the seeds of C. lanatus demonstrated high toxicity in BST at 13.4 and 14.56 µg/ml respectively. It therefore suggests the use of C. lantus seeds for the development of anti-cancer and insecticidal agents.

INTRODUCTION

Cucurbitaceae is a single family in the order *Cucurbitales* with about 110 genera and 640 species; abundant in the tropics, mostly herbs, climbing by tendrils, angiospermic with dicotyledonous seeds and generally unisexual, regular and pentamerous. Typically, include; *Citrullus, Cucurbita, Momordica* and *Cucumis*. *Cucurbits* are a well recognized source of secondary metabolites such as cucurbitacins, tetracyclic triterpenoids. Alkaloids have been reported in *Momordica* and sapponins in *Cucurbita*, *Citrullus* and *Lagenaria*. *Cucurbitaceae* emerged from our food ranking system as excellent sources of vitamin A (in the form of beta carotene), a very good source of vitamin C, potassium, dietary fibre and manganese. Intake of water melon, winter squash and cucumber may protect against diabetic heart disease and may be useful for preventing other complications caused by free radicals often seen in long term diabetes.Concoction of ground seeds of water melon could be used to treat kidney and bladder diseases[1]. Personal communication with water melon farmers during sample collection, revealed the use of water melon seeds by the farmers in treating various ailments, typically piles, kidney infections and gall stones A search for new anti-cancer drugs has taken many different approaches. The brine shrimp lethality bioassay is efficient, rapid and inexpensive tests that require only a

relatively small amount samples. The technique is easily mastered, costs little, and utilizes small amount of test material.[2] has been successively employed for *in-vivo* lethality bioassay-guide fractionation of active cytotoxic and antitumor agents such as trilobacin from the bark of *Asimina* triloba [3]. The present study, sets to test the toxicity of *Citrullus lanatus* plant extracts and fractions on *Artemia salina* (Brine shrimp test).

MATERIALS AND METHODS

Collection and handling of plant materials

Citrullus lanatus plant materials namely fruits, stems and leaves of light bark variety were collected from the water melon plantation site in Karfi, Kura local government, Kano state, Nigeria. The taxonomic identity of the plant materials was confirmed by comparing with voucher specimens No. 2234 at the department of Biological science Herbarium, Ahmadu Bello University, Zaria, Nigeria.

The plant materials (seeds, stems and leaves) were shade dried for two weeks in the Herbarium of the department of biological science Bayero University, Kano-Nigeria. Subsequently, the dried plant materials were separately ground using mortar and pestle. These were stored in dried plastic containers prior to extraction.

Extraction and Fractionation of the Plant Materials

The extraction procedure described by [4] was adopted. The ground seeds, stems and leaves were extracted using 95% ethanol in the ratio of 1:5 (w/v). Therefore, 50 grams of each ground plant materials was percolated with 1.25 liters of 95% ethanol for 1 week at room temperature. The percolates were filtered using whatman No. 1 filter paper to remove solids. The filtrates were separately evaporated to dryness at 40^0C using rotary vacuum evaporator model No. 23777 to obtain ethanolic soluble extracts of seeds (ESS), stems (EST) and leaves (ESL).

The three ethanolic soluble extracts were separately partitioned between chloroform and water in the ratio (100ml, 1:1) with the help of separation funnel.

The chloroform and water soluble fractions were subsequently evaporated to dryness using water bath. The six fractions namely chloroform soluble fraction of seeds (CFS), chloroform soluble fraction of stems (CFT) an chloroform soluble fraction of leaves (CFL) as well as water soluble fraction of seeds (WFS), water soluble fraction of stems (WFT) and water soluble fraction of leaves (WFL) were further partitioned separately with aqueous 90% methanol and petroleum ether (100ml, 1:1) to obtain six fractions namely methanol soluble fraction of seeds (MFS), methanol soluble fraction of stems (MFT) and methanol soluble fraction of leaves (MFL) as well as petroleum ether soluble fraction of seed (PFS), petroleum ether soluble fraction of stems (PFT) and petroleum ether soluble fraction of leaves (PFL). All the extracts and fractions were stored in refrigerator until needed.

Brine Shrimp Lethality Bioassay

The method reported by [2] as experimentally demonstrated by [5] was employed. A quantity (100ml) oceans/sea salt water obtained from Lagos beach, Nigeria, was put into a hatching chamber. A 50mg of *Artemia salina* (Leach) eggs (*Artemia lnc*. California) were added in the hatching chamber. The hatching chamber was kept under fluorescence dgjefbbulb for 72hours, for the eggs to hatch into shrimp larvae. A quantity (20mg) of each test fraction was separately dissolved in 2ml methanol. These served as stock solutions. Using two microlitre syringes (500µg/ml) (0.5ml) and 50µl (0.05ml) capacity respectively, solution of 500, 50 and 5µl was transferred into vials corresponding to 1000, 100 and 10µg/ml respectively. Each dosage was tested in triplicate. The vials (9 per test fraction) and one control, with 500µl of the solvent (methanol) were allowed to evaporate to dryness in about 48 hours, at room temperature.Four milliliter (4ml) of the ocean/sea salt water was added to each vial (with test fraction) and 10 larvae of *Artemia salina* taken 72 hours after the initiation of hatching were added to each vial.The final volume of the ocean/sea salt water in each vial, was adjusted to 5ml, immediately after adding the shrimp larvae. Twenty four hours later, the number of survival shrimp larvae at each dosage was counted and recorded. LC_{50} values were determined with 95% confidence intervals by analyzing the data with a "Finney programme".

Control: A 4ml of the ocean/sea salt water was put into a glass vial. Ten (10) larvae of *Artemia salina* were placed. The final volume of the sea water was adjusted to 5ml. This served as control.

RESULTS AND DISCUSSIONS

Results of Brine Shrimp lethality Bioassay

The brine shrimp lethality bioassay was purposely conducted to determine the level of cytotoxicity of the active substance in the differet plant extracts. The result shows that ethanol soluble extract of seeds (ESS) and water soluble fraction of seeds (WFS) had the lowest LC_{50} values, hence had the greatest cytotoxicity potential in very low concentrations 13.4µg/ml and 14.96µg/ml respectively. The chloroform soluble fraction of leaves (CFL) and chloroform soluble fraction of seeds (CFS) had the highest LC_{50} values 166.1µg/ml and 126.68µg/ml respectively, hence less toxic (Table 1).

Table 1: Brine shrimp lethality bioassay results of *C. lanatus* plant parts

Plants parts used	Extracts/fractions tested	LC_{50} (µg/ml) at 90% confidence interval
Stems	EST	> 1000
	CFT	72.8 (199.1 – 43.3)
	WFT	> 1000
	MFT	> 1000
	PFT	> 1000
Leaves	ESL	39.3 (72.8 – 17.2)
	CFL	166.1 (459 – 72)
	WFL	64
	MFL	> 1000
	PFL	> 1000
Seeds	ESS	13.4 (30.19 – 18.2)
	CFS	126.68 (217.3 – 73)
	WFS	14.96 (83.4 -0)
	MFS	> 1000
	PFS	> 1000

KEY: EST = Ethanol extract of stem; ESL = Ethanol soluble extract of leaves; ESS = Ethanol soluble extract of seeds; CFT = Chloroform soluble fraction of stem; CFL = Chloroform soluble fraction of leaves. CFS = Chloroform soluble fraction of seeds. WFT = water soluble fraction of stem; WFL = water soluble fraction of leaves; WFS = water soluble fraction of seeds; MFT = methanol soluble fraction of stems. MFL = methanol soluble fraction of leaves. MFS = methanol soluble fraction of seeds; PFT = petroleum ether soluble fraction of stems; PFL = petroleum ether soluble fraction of leaves; PFS = petroleum ether soluble fraction of seeds; Seeds = ethanol soluble extract of seeds (ESS) and water soluble fraction of seeds (WFS); LC_{50} = lethal concentration.

The brine shrimp lethality bioassay result is interesting. Essentially this bioassay is useful in the establishment of bioactive substances that can be suitable candidates in treatment of various physical ailments. The ethanol soluble extract of seeds (ESS) and water soluble fraction of seeds (WFS) of *C. lanatus* which indicated greatest cytotoxicity due to the lowest LC_{50} is important. [5] reported on strong larvicidal activity of *Annona squamosa* (BST LC_{50} 3(9-0) µg/ml with desirable selective activity against *Salmonella* species and *Escherichia coli*.

Water melon has been extensively profiled for carotenoid content. Carotenoids have been identified as having anti-oxidant properties that may help prevent cancer, heart disease and slow the ageing process [6].

CONCLUSION

The cytotoxic effects are attributed to the presence of tannins as found in the ethanolic fraction of the seed. The lowest cytotoxic effect demonstrated by the leaves due to the highest LC_{50} confirmed that, the leaves may not be useful in treatment of cancer [7].The seed of *C. lanatus* light bark variety is highly cytotoxic. It therefore supports the use of the seeds in the development of anti-cancer and insecticidal agents.

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