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Original Research Article

Phytochemical screening and cytotoxicity evaluation of *Launaea Cornuta* H. (Asteraceae) using brine shrimp

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Abstract

***Launaea cornuta* is an erect perennial plant with hollow stems up to 1.5 m high and creeping rhizomes and belongs to the family of *Asteraceae* which is the largest family of flowering plants. A single plant can cover a large area as a result of spread by rhizomes*. L. cornuta* can become a dominant weed in a range of semiarid subtropical ecologies. The herb is native to Africa and occurs on alluvial soils in cultivated areas, including irrigated crops, on roadsides, near rivers and bush vegetation. *L. cornuta* is used as a wild vegetable in African communities as source for vitamin C. The decoction is used to treat typhoid, ear pain, stomach pain, chronic join pain, measles, gonorrhea, ascariasis, swollen testicles, warts, diabetes and in the management of breast cancer and benign prostate hyperplasia. The objective of the study was to carryout physical and chemical properties of the plant constituent as well as screening for cytotoxic activity against brine shrimps. The plant materials were collected and dried under shade in the laboratory benches. The ground materials were used for preliminary phytochemical studies and revealed the presence of tannins, flavonoids, alkaloids, glycosides, steroids, coumarins, gums, mucilages, phenols, terpenoids and fatty acids while saponins, volatile oils and phlobotanins were absent. Extraction was achieved by sequential maceration using petroleum ether, chloroform and methanol respectively. Aqueous extract was prepared separately. The organic solvent extracts were dried in *vacuo* while the aqueous extract was lyophilized. The brine shrimp lethality tests were carried out and the petroleum ether and chloroform extracts exhibited moderate cytotoxicity against brine shrimp with LC_{50} value of 373 µg/ml and 342.7 µg/ml respectively. The varied groups of chemicals reported in this herb suggestively indicate lay scientific evidence for the herb use in various ailments and moderate toxicity against brine shrimp is preliminarily justify the reason for embarking on evaluating the herb fractions for bioactivity.**

Keywords: Bioactivity, LC_{50}, Maceration, Toxicity

INTRODUCTION

Launaea cornuta belongs to the family asteraceae (Compositae) which is one of the eight families of the order campanulales. Asteraceae is the largest family of the flowering plants and contains about 960 genera and some 13,000 species (Evans, 2009). Compared with some large families such as *leguminosae*, the number of important economic products derived from this family is relatively small. Chemical research in recent years has

increased medical interests in the family and there is a better knowledge of many almost-discarded folk remedies as well as hitherto uninvestigated plants. The latter include some having antitumor or antibacterial activity and others forming commercial sources of rubber latex (Evans, 2009) Stevioside, anent-kaurene glycoside from *Stevia rebaudiana* is used as a sweetener for soft drinks in a number of countries and also as antidepressant.

Launaea cornuta is an erect perennial herb with milky juice and hollow stems up to 1.5 m height and creeping rhizomes. Leaves are deeply divided form a rosette at the base, alternate on the stem, sessile, up to 2.5 cm long by 3 cm wide, entire or with two to three pairs of lobes acute-pointed near the base. Inflorescence large, diffuse with numerous yellow flower heads on peduncle about 2.5 cm long involucres up to 10 cm long by 4 mm cross, glabrous or shortly pubescent, phyllares in two to three rows, 2-4 mm long outside, up to 10 mm long inside. Florets 10-25, yellow up to 15 mm long, ligules often reddish outside seeds pale brown, elliptical, ribbed 2-4 mm long with white pappus 5 mm long, (Ashirley 1994; Maundu *et al*., 1999). The herb is native to Africa and commonly known as wild or bitter lettuce, moleita and merlot (Sudan), muthunga (Kikuyu) muthunga (Meru and Embu) mchunga (Swahili), Mnyinya (Taita), and Achak (Luo). It occurs on alluvial soils in cultivated areas, including irrigated crops, on roadsides, near rivers and bush vegetation. A single plant can cover a large area because of spread by rhizomes. It is the commonest species of *Launaea* around Nairobi, Kenya (Ashirley 1994; Maundu *et al*., 1994).

The biological activities of *L. cornuta* that have been reported recently are the hypoglycaemic activity and safety of the ethylacetate extract of *L. cornuta* shrub (Karau *et al*., 2014). Ethnomedically *L. cornuta* is used as a wild vegetable in African communities for instance Kilifi, the coastal region of Kenya and Nigeria as a source of vitamin C (Schippers, 2004). In East Africa the decoction is used to treat typhoid, the leaves juice is dripped in the ear to stop pain and the herb is boiled with water and the extract used to wash the body for the treatment of measles. In Tanzania the leave decoction is used in the treatment of gonorrhea, ascariasis, stomach pains and fresh roots are chewed to cure swollen testicles (Kokwaro, 2009). In Kenya, its good browse for goats and rabbits. The roots are used for the treatment of warts and also administered orally in the management of chronic joint pains (Wambugu *et al*., 2011) and the concoction used to manage breast cancer and benign prostate hyperplasia diabetes (Kareru *et al*.,2007). The objective of this study is was to carry out preliminary phytochemical screening and evaluation of bioactivity of *L. cornuta* aqueous and organic solvent extracts using brine shrimp lethality assay.

MATERIAL AND METHODS

Collection and processing of plant material

Fresh plant material (whole plant) was harvested at Kasarani area, Nairobi County, on 14th August 2012 and was authenticated by Mr. Geoffrey Mungai of the National Museum of Kenya. The voucher specimen was identified, prepared and labeled with the number JM- 01- 2012 and was deposited at Mount Kenya University herbarium at the School of Pharmacy. The plant materials were shade dried in a properly ventilated room for one week. Garbling was done to remove unwanted impurities such as grass, soil, and other extraneous plant materials. The aerial parts were separated from roots by cutting and then were dried separately and later were cut into smaller pieces and ground into moderately coarse powder.

Phytochemical investigation

Chemical tests were employed in the preliminary phytochemical screening for various secondary metabolites such as alkaloids (Mayer's and Drangedorff's reagents),cardiac glycosides (Kedde and Keller-Killian tests), saponins glycosides (frothing and haemolytic tests),anthracene glycosides (Borntrager's test for combined and free anthraquinones), cyanogenetic glycosides (sodium picrate paper test), steroids (Salkowski test), phenols (ferric chloride test), flavonoids, tannins, coumarins, volatile oils (smell test) and carotenoids (antimony trichloride test) (Anjali and Sheetal, 2013, Chugh, *et al*., 2012; Trease and Evans, 2008). The results for phytochemical screening were as indicated in table 1.

Extraction of Plant materials

Approximately 100 g of the plant materials were sequentially macerated using pet ether, chloroform and methanol at room temperature and after 48 h filtered through Whatman number 1 filter paper. The filtered extracts were reduced partially in *vacuo* at 40 ^{0}C and thereafter into complete dryness in an oven set at 35 ^{0}C. The water extract was prepared by weighing approximately 20 g of each plant part and heated with enough water to boil. The boiling was allowed for 5 min and the extract cooled at room temperature after which, it was filtered and then freeze dried (Harborne, 1976; Tiwari *et al*., 2011).

The Brine shrimp lethality test

The brine shrimp lethality test (BST) was used to predict the presence in the extracts of cytotoxic activity.

Table 1. Qualitative phytochemical analysis of crude extract of *Launaea cornuta*

Phytochemicals/Test	Part of the plant	
	Roots	Aerial
Alkaloids		
1. Mayer,s	+	+
2. Drangedorff	+	+
Cardioglycosides (Keller-killan)	+	+
Anthraquinone glycosides		
3. Borntragers	+	+
4. Modified Borntragers	+	+
Saponins	-	-
Tannins	+	+
Phenols	+	+
Steroids	+	+
Coumarins	-	-
Flavonoids	+	+
Volatile oils	-	-
Fatty acids	+	+
Gums and mucilages	+	+
Terpenoids	+	+
Phlobotannins	-	-

Solutions of the extracts were made in DMSO and brine solution to a volume of 5 ml at concentrations of 1000 µg/ml, 100 µg/ml and 0 µg/ml in triplicates. Ten brine shrimp larvae were placed in each of the triplicate vials. After 24 h. the nauplii were examined against a lighted background and the average number of the surviving nauplii in each test tube was determined and the obtained data was recorded and analyzed for LC_{50} using Graphed prism version 4 programme.

Data analysis

The brine shrimp lethality test assay results obtained were analysed using the Graphed Prism version 4 Program and were illustrated as shown in Table 3.

RESULTS AND DISCUSSION

(a) Qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis of *L. cornuta* crude extract showed presence of flavonoids, coumarins, alkaloids, glycosides, steroids and tannins. However, saponins were absent in the crude extract. This work confirms some of the work done early on this plant but differ on the report of presence of saponins (Musila *et al*., 2013, Karau *et al*., 2014-b).

The alkaloids have been associated with medicinal uses for centuries and one of their common properties is cytotoxicity but also analgesic, antispasmodic and antibacterial activities have been reported. The glycosides are known to lower blood pressure and also especially cardiac glycosides have been used for over two centuries as stimulants incase of cardiac failure. The anthraquinone glycosides have also been used as laxatives. Steroids have antibacterial properties and they are also very important compounds especially due to their relationship with compounds such as sex hormones.

The phenolic compounds that are coumarins, flavonoids and tannins are the largest and most ubiquitous groups of plant metabolites. They posses biological properties such as antiaptosis, antiaging, anticarcinogen, antiinflammation, antiartheriosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activities. This preliminary phytochemical screening would perhaps justify the traditional uses of the plant in management of pain, cancer, diabetes and infectious diseases.

(b) The brine shrimp lethality test represents a rapid, inexpensive and simple bioassay for testing the *L. cornuta* extracts for activity which in most cases correlates reasonably well with cytotoxic, anti-tumor and insecticidal activity. LC_{50} values (373-1283 µg/ml) of the plant extracts were obtained and were recorded as indicated in table 2 below.

Based on the obtained data of LC_{50} values, the solvent extracts from aerial part of *L. cornuta* are classified according to the strength of toxicity to express their outright toxicity or presence of potential anticarcinogenicity (Table 3)

Table 2. Table showing LC_{50} values of different solvent extracts of *L. cornuta* aerial part

Solvent Extracts	LC_{50} Values
Petroleum Ether	373 µg/ml
Chloroform	342.7 µg/ml
Methanol	970.6 µg/ml
Aqueous	1283 µg/ml

Table 3. Classification of toxicity of *L. cornuta* extracts of aerial part

LC_{50} values	Classification	Extract
<1 µg/ml	Extremely toxic	None
1-100 µg/ml	Very highly toxic	None
100-200 µg/ml	Highly toxic	None
200-500 µg/ml	Moderately toxic	Petroleum ether and Chloroform Extracts
500-1000 µg/ml	Lowly toxic	Methanol extract
>1000 µg/ml	Particularly non-toxic	Water extract

The brine shrimp test results indicate that the petroleum ether and chloroform extracts had LC_{50} values of between 200-500 µg/ml which suggests that they are moderately toxic while the methanolic extract had LC_{50} values of between 500-1000 µg/ml and this suggests low toxicity. On the other hand the aqueous extract had LC_{50} which was greater than 1000 µg/ml and therefore interpreted as particularly non –toxic though earlier reports indicate that the LC_{50} of chloroform: methanol (1:1) and aqueous leave extracts were 74 µg/ml or 75.26 µg/ml and 842 µg/ml or 341 µg/ml respectively (Nguta *et al*., 2011, Musila *et al*., 2014) and therefore classifying both the organic and aqueous extracts as toxic as opposed to this study where the aqueous extract is considered non toxic. Based on the basic premise that pharmacology is simply toxicology at a lower dose and toxicology is simply pharmacology at a higher dose, in this study the results indicate that none of the extracts are severely toxic considering that there were no activity against the brine shrimps up to concentrations of up to 200 µg/ml. In the African traditional medicine practices, *L. cornuta* is used as an aqueous extract and therefore this result reveal that with LC_{50} greater than 1000 µg/ml then the aqueous extract would be safe and non-toxic when used as such.

The extracts with moderate and low toxicity that is petroleum ether, chloroform and methanol extracts can be fractionated for isolation of bioactive compounds especially for cytotoxicity fractions or compounds.

CONCLUSION AND RECOMMENDATION

The presence of various phytochemicals constituents such as glycosides, glycosides, steroids, tannins, flavonoids and coumarins in the aerial parts of *L. cornuta* and the results for brine shrimp lethality test confirms that this plant is safe to use as medicine and can be a potent source for complementary/alternative medicines and modern medicine too. The preliminary phytochemical screening bioassay studies using brine shrimp paves way for ethnobotanical and pharmacological investigations for the future discovery of new sources of drugs. The results from this work and other related studies recommend the traditional practice of using *L. cornuta* and it is suggested that further work on bioassay-guided isolation should be carried out to isolate, purify and characterize the active constituents of *L. cornuta*. In addition it is encouraged to elucidate the possible mechanism of action as well as studies on chronic toxicity of the *L. cornuta* extracts.

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