



Full Length Research Article

PHYTOCHEMICAL SCREENING AND BRINE SHRIMP LETHALITY ASSAY OF THE LEAF EXTRACTS OF *CUCURBITA MAXIMA*, *EUPHORBIA HIRTA*, *LEPTADENIA HASTATA* AND *MITRACARPUS SCABER*

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Accepted 17th April 2016; Published Online 31st May 2016

ABSTRACT

Soxhlet apparatus was used to obtain leaf extracts of *Cucurbita maxima*, *Euphorbia hirta*, *Leptadenia hastata* and *Mitracarpus scaber* using chloroform and ethanol solvents while maceration was used for the aqueous extraction. Phytochemical screening was performed on the leaf extracts which revealed the presence quite a number of phytochemicals. Brine shrimp Lethality Assay (BSLA) was carried out using the various extracts. The observed cytotoxicity was expressed in terms of LC₅₀ (lethality concentration). The surviving brine shrimps were recorded after 24 hours. The results revealed that the extracts showed potent activity against the brine shrimps having LC₅₀ values less than 1000ppm. This possibly indicates the presence of biologically active components in these extracts which could be responsible for the known pharmacological effects of the plants. Thus, the results of the study further highlights the ethnotherapeutic importance of the leaves of these plants.

Key words: Phytochemical, Brine shrimp Lethality Assay, LC₅₀, Plant extracts.

INTRODUCTION

Plants have been used over the years as curative agents against many infections and have been exploited in traditional medicine with their curative potentials well documented (Dubey *et al.*, 2004; Ibrahim *et al.*, 2011). Most plants are capable to synthesize some chemical compounds that are useful to man and animals. These compounds are able to perform physiological action in the body. These chemical compounds are known as phytochemicals. These phytochemicals have important health benefits and can also be used to treat infections especially of microbial origin (Ranjaragan and Sathiyavani, 2014). There are two basic classes of phytochemicals namely primary and secondary metabolites. The primary metabolites comprise of common sugars, amino acids, proteins and chlorophyll while the secondary metabolites consist of alkaloids, flavonoids, tannins and so on (Kubmarawa *et al.*, 2007; Edeoga *et al.*, 2005). Screening of the phytochemical components in a given plant extract is aimed at identifying the nature of the compound present which has been reported to be responsible for the observed medicinal action. These compounds exact their effect by destroying the microorganisms responsible for the infection, clearing up residual symptoms, improving immunity and plays vital role in reducing man's ageing process (David *et al.*, 1997).

Cucurbita maxima (pumpkin) is an edible and highly medicinal plant which possesses trifoliate leaves with flexible stems (Kirtikar and Basu, 2003). The plant has been used traditionally as medicine in many countries around the world (Popovic, 1971; Xia, *et al.*, 2004; Adolfo and Michael, 2005). Traditionally, it is used in most countries as curative agents for diabetes, hypertension and microbial infections (Caili *et al.*, 2006). *Euphorbia hirta* which is classified under the family Euphorbiaceae, is a small annual herb found in most parts of the world (Patil *et al.*, 2009; Ogueke *et al.*, 2007; Soforowa, 1982). The plant has been widely used in treating many respiratory and skin infections. It is used in India to treat dysentery, jaundice, pimples and worm infestations in children (Kirtikar and Basu, 1991). In Nigeria, it is used to treat boils, sores and promotes wound healing (Igoli *et al.*, 2005).

Leptadenia hastata is an edible non-domesticated valuable herb with creeping latex stems, glabrous leaves, glomerulus and racemes flowers as well as follicle fruits. It is typically grown in tropical dry lands in sandy soil. In Chad, the roots are used to treat scabies (Betti *et al.*, 2011). This plant is commonly used in Hausa-speaking communities in Nigeria as a spice and used in sauces (Ibrahim *et al.*, 2012). Also in Nigeria, local healers use the plant for hypertension, catarrh and skin diseases (Dambatta and Aliyu, 2011). In Burkina Faso, it is used locally for sexual potency by chewing the leaves. Decoction of the leaves is used in the treatment of trypanosomiasis. It is also useful in the treatment of skin diseases and in wound-healing by the application of its latex. *Mitracarpus scaber* belongs to the family Rubiaceae. The family consists of about 500 genera

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and 6000 species distributed all over the world. The leaves are short and green with parallel lines. The parts of the plant that are normally used are the aerial part and the leaves (Gill, 1992). The plant is known to have effective antifungal and antibacterial properties (Van-wyk *et al.*, 1997; Gupta *et al.*, 1998). The juice of the plant is applied to ring worm and other fungal diseases. The crushed leaves are used as dressing for fresh cuts, wounds and ulcers. The leaf extracts of *Mitracarpus scaber* is widely used in traditional medicine practices in West Africa for the treatment of headache, toothache, amenorrhoea, dyspepsia, hepatic diseases, venereal diseases as well as leprosy.

Some of the traditional medicine involves the use of crude plant extracts which may contain an extensive diversity of molecules, in which most times the biological effects are unknown (Konan *et al.*, 1997) and in most cases, available information regarding the medicinal potential of these plants is not provided with credible scientific data. However, this has prompted several researches to conduct tests to determine the toxicity of medicinal plants (Sasidharan *et al.*, 2009). A general bioassay that appears capable of detecting a broad spectrum of bioactivity present in plant crude extracts is the Brine Shrimp (*Artemia* sp.) Lethality Assay (BSLA) (Pisutthanan *et al.*, 2004). BSLA is used as an indicator for general toxicity and also as a guide for the detection of antitumor and pesticidal compounds (Meyer *et al.*, 1982). The low cost and ease of performing the assay and the commercial availability of inexpensive brine shrimp eggs makes BSLA a very useful bench top method (McLaughlin *et al.*, 1991). This assay has been recommended as being important in isolating the bioactive compounds from plant extracts (Sam, 1993). The aim of this work is to investigate the phytochemical components of the leaf extracts of the selected plants as an additional scientific assessment of their therapeutic potency.

MATERIALS AND METHODS

Sample Collection

Fresh leaves of *Curcubita maxima*, *Euphorbia hirta*, *Leptadenia hastata* and *Mitracarpus scaber* were obtained from around the old campus of Bayero University, Kano, Nigeria. The taxonomic identities of these plants were confirmed in the Department of Biological Sciences, Bayero University, Kano. The plants collected were washed with distilled water to remove the soil and dust particles. They were thoroughly air-dried, powdered and stored in airtight containers until required.

Extraction Procedure

The extraction was done using a 250ml capacity soxhlet apparatus in which 25g of the powdered leaves of each plant was extracted separately using chloroform and ethanol as the organic solvents while the aqueous extracts were obtained using maceration method, though 5% H₂SO₄ was added to prevent fermentation.

Phytochemical Analysis

Each extract was investigated for the presence of secondary metabolites like alkaloids, flavonoids, reducing sugars,

saponins, steroid, tannins, phytosterols and cardiac glycosides, using the qualitative standard method according to Harborne (1983), Trease and Evans (2002) and Sofowora (1993) and Roopalathau and Vijay (2013). The colour intensity of the extracts and/or the appearance of solids during the identification reactions allow a semi-quantitative evaluation of the presence of secondary metabolites in them.

Test for Alkaloids

To 0.1ml of the extract and fractions in a test tube, 2-3 drops of Dragendoff's reagent was added. An orange red precipitate with turbidity denotes the presence of alkaloids.

Test for Flavonoids

To 4mg/ml of the extracts and fractions, a piece of magnesium ribbon was added followed by a drop-wise addition of concentrated HCl. A colour change from orange to red indicates the presence of flavones; red to crimson indicates presence of Flavonoids.

Test for Reducing sugars

To 1ml of extract and fraction in separate test tubes, 2.0mls of distilled water was added followed by addition of Fehling's solution (A+B) and the mixtures warmed at 40°C. Appearance of brick red precipitate at the bottom of the test tube indicates the presence of reducing sugar

Test for Saponins

Half gram of the powdered leaf was dispensed in a test-tube and 5.0ml of distilled water was added and shaken vigorously. A persistent froth that lasts for about 15 minutes indicated the presence of saponins.

Test for Steroids

Two milliliters of the extracts was be evaporated to dryness in separate test tubes and the residues dissolved in acetic anhydride followed by addition of chloroform. Concentrated sulphuric acid was added by means of a pipette via the side of the test tubes. Formation of brown ring at the interface of the two liquids and violet color in the supernatant layer denotes the presence of steroids.

Test for Tannins

Two milliliters of the extract was diluted with distilled water in separate test tubes, 2-3 drop of 5% ferric chloride (FeCl₃) solution would be added. A green-black or blue colouration indicates tannins.

Test for Phytosterol

Two miligrams (2mg) of the extract was dissolved in 2ml of acetic anhydride, heated to boiling, cooled and then 1ml of concentrated sulphuric acid was added along the side of the test tube. Formation of a brown ring at the junction and the turning of the upper layer to dark green colour confirms the test for phytosterols.

Test for Cardiac glycosides

Zero point four (0.4) ml of glacial acetic acid and a few drops of 5% ferric chloride solution was added to a little of the dry extract. Further 0.5ml of concentrated sulphuric acid was added along the side of the test tube carefully. The formation of a blue colour in acetic acid layer confirms the presence of cardiac glycosides.

Brine Shrimp Lethality Assay (BSLA)

Brine shrimp lethality bioassay was used to determine the toxicity of the extracts to simple zoological organism (*Artemia salina*) as described by Randhawa, (2009) and Lilybeth and Olga, (2013). Brine shrimp eggs (Sera, Heidelberg, Germany) used for this work were collected from the Department of Chemistry, BUK. Filtered, artificial seawater was prepared by dissolving 38.0g of sea salt in 1.0 liter of distilled water for hatching the shrimp eggs. The seawater was put in a small plastic container (hatching chamber) with a partition for dark (covered) and light areas. Shrimp eggs were added into the dark side of the chamber covered with aluminum foil while the lamp above the other side (light) will attract the hatched shrimp. Two days were allowed for the shrimp to hatch and mature as nauplii (larva). A dilution procedure developed by McLaughlin and Rogers, (1998) was adopted in the preparation of the different dilutions of the plant extracts for BSLA where 20.0 mg of each extract was dissolved in 2.0 ml of the solvent. The final concentrations were 1000, 100, 10 ppm ($\mu\text{g/ml}$).

The number of surviving shrimps were counted and recorded after 24 hours. The nauplii were considered dead if no movement of appendages were observed within 10 seconds. Using probit analysis, the lethality concentration (LC_{50}) was assessed at 95% confidence intervals. LC_{50} value of less than 1000 $\mu\text{g/ml}$ is toxic while LC_{50} value of greater than 1000 $\mu\text{g/ml}$ is non-toxic (Owolarafe *et al.*, 2014). The percentage mortality (%M) was also calculated by dividing the number of dead nauplii by the initial number of live nauplii used, and then multiplied by 100%. This is to ensure that the death (mortality) of the nauplii is attributed to the bioactive compounds present in the plant extracts.

RESULTS AND DISCUSSION

The results of the phytochemical screening of the various plant extracts revealed the presence as well the absence of some of the various phyto-constituents in the leaf extracts (Table 1). The result showed that the plants have quite a number of chemical constituents, which may be responsible for the many pharmacological actions. Although their specific roles were not investigated in this study. Other researchers have reported the presence of phytochemicals in the leaf, pericarp and seed of *C. maxima* (Ravishankar *et al.*, 2012; Attarde *et al.*, 2010; Moumita *et al.*, 2011). The Phytochemical components of *Eubhorbia hirta* have been established in previous studies and these include tannins, saponins, alkaloids, carbohydrates, phenols, flavonoids, sterols (Vasanth and Khatoun, 2013; Onawumi *et al.*, 2012).

Table 1. Phytochemical constituents of the various plant extracts

Phytochemicals	<i>Cucurbita maxima</i>			<i>Euphorbia hirta</i>			<i>Leptadenia hastata</i>			<i>Mitracarpus scaber</i>		
	Ch.	Et.	Aq.	Ch.	Et.	Aq.	Ch.	Et.	Aq.	Ch.	Et.	Aq.
Saponins	+	-	-	-	-	+	+	-	+	+	-	+
Flavonoids	+	+	+	+	+	+	-	+	+	+	+	+
Reducing sugars	-	-	-	+	+	-	-	+	-	+	+	+
Tannins	+	+	-	-	+	-	-	+	-	+	+	+
Steroids	+	-	-	+	+	-	-	-	+	+	+	+
Phytosterols	-	+	-	+	+	-	+	+	-	+	+	-
Cardiac glycosides	+	+	-	-	+	-	+	+	-	+	-	-
Alkaloids	+	+	-	-	-	-	-	+	+	+	-	+

Key: Chloroform Extract=(Ch) Ethanol Extract=(Et) Aqueous Extract=(Aq)
Absent=(-)Present=(+)

Table 2. LC50 values of the various extracts from the BSLA

PLANT EXTRACT	LC ₅₀ Value
<i>C. maxima</i> (Chloroform)	97ppm
<i>C. maxima</i> (Ethanol)	276ppm
<i>C. maxima</i> (Water)	296ppm
<i>E. hirta</i> (Chloroform)	239ppm
<i>E. hirta</i> (Ethanol)	118ppm
<i>E. hirta</i> (Water)	256ppm
<i>L. hastata</i> (Chloroform)	148ppm
<i>L. hastata</i> (Ethanol)	70ppm
<i>L. hastata</i> (Water)	294ppm
<i>M. scaber</i> (Chloroform)	125ppm
<i>M. scaber</i> (Ethanol)	104ppm
<i>M. scaber</i> (Water)	153ppm

There were three (3) replicates in each concentration. A control test was also prepared. After two days, when the shrimp larvae are ready, 4.0 ml of the artificial seawater was added to each test tube and 10 brine shrimps were introduced into each tube. Thus, there were a total of 30 shrimps per dilution. Then the volume was adjusted with artificial seawater up to 5.0 ml per test tube. The test tubes were left uncovered.

Similarly, Sanda *et al.* (2013) and Muhammad and Zubaida (2015) also reported the presence of phytochemicals in the leaves and roots of *L. hastata* while Shikafi (2013) reported the presence of phytochemicals in the leaf extracts of *M. scaber*. The presence of these bioactive compounds in these plant materials have been associated with antimicrobial activities. The presence of these secondary metabolites in

plants, produce some biological activity in man and animals and it is responsible for their use as herbs. These compounds also serve to protect the plant against infection by microorganisms, predation by insects and herbivores, while some give plants their odours and/or flavours and some still are responsible for their pigments (ketkar and Ketkar, 1995). The results of the brine shrimp lethality assay are shown in Table 2. The chloroform, ethanol and aqueous extracts of *C. maxima* showed LC₅₀ values of 97ppm, 276ppm, 211ppm respectively. Also, the chloroform, ethanol and aqueous extract of *E. hirta* showed LC₅₀ values of 239ppm, 118ppm, 256ppm respectively. The LC₅₀ values for *L. hastata* showed 148ppm, 70ppm, 294ppm while that of *M. scaber* showed 125ppm, 104ppm, 153ppm for chloroform, ethanol and aqueous respectively. The leaf extracts of *C. maxima*, *E. hirta*, *L. hastata* and *M. scaber* exhibited good brine shrimp larvicidal activity. The observed lethality of the four plant extracts to brine shrimps indicated the presence of potent cytotoxic, bioactive and probably antitumor components of these plants. Cytotoxic action of a drug is believed to be provided by disturbing the fundamental mechanisms associated with cell growth, mitotic activity, differentiation and function (Goodman, *et al.*, 1980). The observed cytotoxic activity for these extracts may be due one of these mechanisms. Hence, the ethno-pharmacological activities of these plant species might be due to the different bioactive compounds present in these plants. According to Owolarafe *et al.* (2014) and Meyer *et al.* (1982), crude plant extract is active if it has an LC₅₀ value of less than 1000 ppm or µg/mL while it is considered inactive if its LC₅₀ value is greater than 1000 ppm or µg/mL. From the result, all the plant extracts possessed cytotoxic activity against the brine shrimp and considered as containing active or potent components. This is because their LC₅₀ values are less than 1000 ppm. Although, the Brine Shrimp Lethality Assay (BSLA) is inadequate to determine the mechanism of action of the various bioactive component present in the different plant extracts used in this study.

Conclusion

The phytochemical screening of the leaf extracts of *Curcubita maxima*, *Euphorbia hirta*, *Leptadenia hastata* and *Mitracarpus scaber* revealed the presence of some phytochemical compounds. These extracts exhibited cytotoxic activity against brine shrimp. Hence, it is considered as containing active or potent components with LC₅₀ values less than 1000 ppm or µg/mL. From this result, it is evident that the leaves of the plants used in this study may have curative properties against several human pathogens and suggest its importance in traditional medicine.

Recommendation

Efforts should be devoted to characterizing the bioactive components of these plants that could be used in the synthesis of more useful drugs.

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