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Full Length Research Paper

Phytochemical properties and toxicity to brine shrimp of medicinal plants in Erute county, Lira district, Uganda

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Erute county is known to have a number of medicinal plants that are used commonly as remedies of many ailments. The study was conducted in 54 parishes of Erute county and aimed at determining the phytochemical constituents and toxicity of the medicinal plants used by the community. Questionnaires, semi-structured interviews and focused group discussions were used to obtain ethnobotanical data of these plants. Field visits were made with traditional medicinal practitioners (TMPs) and eleven key medicinal plants (*Helicrysum gerberifolium* Sch.BIP*, Pseudocedrela kotschyi* (Sceinf.) Harms*, Clematis hirsute* Perr. and Gull.*, Clerodendrum myricoides* R.B*, Stagnotaenia areliacea* Hosct*, Gladiolus psitticinus* L.*, Trichilia emetica* Vahl*, Schkuria piñata* (Lam.) O. Oktze*, Dombeya kirtii* Mast*, Rhyncosia densifolia* and *Vernonia brachycalyx* Hoffm.) collected and identified. Voucher specimens were deposited in the Herbarium of Botany Department, Makerere University. Roots for chemical analysis were collection, air-dried under mild sunlight and grinded into uniform powder using a metal mortar. The extracts were obtained by successively soaking 100 g in ether, methanol and finally water. The analyzed extracts showed the presence of steriods / terpenoids, alkaloids, flavanoid, coumarin and emodol. Toxicity to brine shrimp was analyzed to determine their ED_{50s} using Finney probability analysis software. The ED50s were in the range of 0.0894 to 2.6045, 0.1108 to 3654 and 0.0298 to 1.8219 for ether, methanol and water respectively. Further extraction with other solvents is necessary for confirmation hence enhancing specific toxicity test.

Key words: Phytochemical, toxicity, brine shrimp, medicinal plants, Erute.

INTRODUCTION

Medicinal plant is defined by many authors as any plant that contains substances or precursors that can be used for therapeutic purposes, synthesis of useful drugs, suppressing, preventing or curing many forms of diseases both in people and their domestic animals (Kokwaro, 1976; Sofowara, 1993; WHO, 2008). Medicinal plants are of great importance to the health of individuals and communities (Edeoga et al., 2005). The use of medicinal plants in traditional medicine has been noted as part of the art of healing based on complex combinations of divinations, magic and rational elements rooted in empherical knowledge of physiological activities of various living things especially plants (Lejoly et al., 1996).

Several authors have emphasized on the importance of traditional medicine in healthcare (WHO, 1978; UNESCO, 1994; Okwu, 1999, 2001; Oryema et al., 2010; Edeoga et al., 2005). Farnsworth and Soejart (1991)

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observed that, there are several plants with known medicinal applications in various cultures. According to Alluri et al. (2005), the importance of medicinal plants in solving healthcare problems in the world is gaining increasing attention. For example, in Malaysia 2000 species from 14 flowering plants have been reported to contain medicinal properties and many have been scientifically proven (Jaganath and Ng, 2000).

Alluri et al. (2005) highlighted that, all medicinal preparations are derived from plants in simple raw plant material or in the refined forms of crude extracts, mixtures and others. Lejoly et al. (1996) stated that, the use and particularly the choice of remedial plants are not borne of systematic research but stems from chance discoveries and from knowledge passed down from generation to generation. These discoveries are scattered in space and in time and so amazingly that people who have no contacts with each others may use the same plant for relieving the same disorder (Lejoly et al., 1996).

Kokwaro (1993) noted that, basic compounds associated with plants are reported to have medicinal values. Lejoly et al. (1996) also reported that, most of the products discovered by man to remedy illness and to dress wounds are all derived from plants and that they are the storehouse of alkaloids, sugars, saponins, aromatic oils, resins and many other medically useful chemicals. Rodríguez and West (1995) also advanced that, the primary sources of chemical compounds that exhibit the novel pharmacological activities essential for new drug developments, are marine invertebrates and vascular plants. Arora et al. (2003) acknowledged that, most traditional medicinal preparations and formulations have been found to be reservoirs of pharmaceuticals. Gurib et al. (2005) for example stated that, plant-derived compounds offer potential source of new antimicrobial, anticancer, and anti-HIV agents among other pharmaceuticals. Some bioactive compounds in plants can also provide the chemical understanding for the treatments of modern plagues caused by pathogenic organisms (Rodriguez and West, 1995).

Samuelson (1988) revealed that, chemical tests are the basis for phytochemical screening. Harbone (1973) however, warned on the risk of the use of such simple standard chemical tests, for they could produce false positive reactions. He for example cited that, Dragendorff's tests's for alkaloids could give a false positive reaction in some non alkaloidal extracts. According to Sofowara (1993), the modes of action of the whole or plant parts producing the biological effect can better be investigated if the principle is characterized. Alluri et al. (2006) forwarded that, the use of plants, plant extracts and pure compounds isolated from natural sources, provide the foundation to modern pharmaceutical compounds. He argued that, the well known Indian systems of Medicine, namely the Ayurveda, Siddha and Urani use predominantly plant base raw materials.

METHODOLOGY

Plant identification

Erute county was purposively selected for the study because of the popular use of medicinal plants for treating various ailments (Oryema et al., 2010). Questionnaire, semi-structured interviews and focused group discussions were used to obtain the ethnobotanical data on these plants. Field visits were made with traditional medicinal practitioners (TMPs) and eleven key medicinal plants which were commonly mentioned by the local were collected and identified by the authors. The voucher specimen were brought and identified by comparing with authenticated specimens in the herbarium of Botany Department, Makerere University.

Extractions

The roots of the key plants were collected, dried on mild sunlight and pounded in a powered form. The pounded plant stuff were extracted successively with ether, methanol and finally with water (Table 3). The powdered plant materials were soaked first with ether solvent for 24 h then filtered. The filtrate was then extracted using a water bath and ether evaporated to dryness. The ether extracts was then scooped and put in a small bottle ready for analysis. The residue from ether was soaked in alcohol for 24 h and later filtered. The iltrate was evaporated to dryness using a water bath. The extract was then removed and bottled for analysis. Finally, the residue from the alcohol solvent was soaked in water for the same period then filtered. The filtrate was put in a round bottomed flask, and evaporation done using water rotary evaporator. The extract was then stored for analysis. This process was repeated for all the eleven different plant materials prepared for analysis.

Qualitative chemical determination of the extracts

The compositions were determined using simple qualitative methods of Sofowora (1984) and Harborne (1993). The separation of the main chemical constituents was obtained through successive and selective extractions of the products with solvents of different polarities (Table 2). The extracts were separately analyzed according to the physical properties of each group of active principles.

Brine shrimp lethality (BST)

The type of bioassay used was that developed by Mclaughlin et al. (1988). The artificial sea water was prepared by dissolving sodium chloride (15 mg), poasium chloride (0.45 g), calcium chloride (0.55 g) and magnesium sulphate (1.76 g) and diluting to make 0.5l of solution. Brine shrimp nauplii hatching tank was filled with water and the eggs sprinkled in to the covered part of the tank. A lamp was placed on the uncovered part of the tank to attract the hatched shrimps. The eggs were left for 24 h to hatch as mature nauplii. Triplicate vials were prepared for each crude extracts. The samples were dissolved in the respective solvents in which they are soluble. Different samples to be tested were introduced in each vials and all were mixed with sea water as food for the nauplii. For each vial, ten nauplii were introducing using pipettes and left for 24 h. The numbers of survivors were counted for each concentration and mortality rates determined. The data was analyzed using Finney

Table 1. Analyzed phytochemicals.

Species	Extract	Ster/ Terp	Alk	Flav	Coum	EM
H. gerberiforlium Sch.BIP	Ether	+	-	-	-	-
	Methanol	+	-	-	-	-
	Water	+	-	-	-	-
P. kotctchyi (Sceinf.) Harms	Ether	+	-	-	-	-
	Methanol	-	-	-	-	-
	Water	+	-	-	-	-
C. hirsute Perr. and Gull	Ether	+	-	-	-	Nt
	Methanol	+	-	-	-	-
	Water	+	-	-	-	-
C. myriciodes RB	Ether	+	-	-	+	-
	Methanol	+	-	-	+	-
	Water	+	-	-	+	-
S. areliacea Hosct	Ether	+	-	-	+	-
	Methanol	+	-	-	+	-
	Water	+	-	-	+	-
G. psitticinus L	Ether	+	-	-	+	-
	Methanol	-	-	-	+	-
	Water	-	-	-	+	-
T. emetica Vahl	Ether	+	-	-	+	-
	Methanol	+	-	-	-	-
	Water	+	-	-	-	-
S. pinnata (Lam).O.Oktze	Ether	+	-	-	-	-
	Methanol	+	+	-	+	-
	Water	+	-	-	-	-
D. kirtii Mast	Ether	+	-	-	+	-
	Methanol	+	-	-	-	-
	Water	+	-	-	-	-

Table 1. Contd.

	Ether	+	-	-	-	-
R. densifolia	Methanol	+	-	-	-	-
	Water	+	-	-	-	-
	Ether	+	-	-	-	-
V. brachycalyx Haffm	Methanol	+	-	-	-	-
	Water	+	-	-	-	-

Not detected (-), Detected (+), Not tested (N), = Alkaloids (Alk), Coumarins (Coum), Flavinoids (Flav) and Emodol (Em).

computer program to determined the ED_{50} values and 95% confidence intervals (Table 2).

RESULTS

Chemical quantitative analysis was carried out on the extracts of the eleven plant species (*H. gerberifolium* Sch. BIP, P. *kotschyi* (Sceinf.) *Harms, C. hirsute* Perr. and Gull., *C. myricoides* R.B., *S. areliacea* Hosct, *G. psitticinus* L, *T. emetica* Vahl, *S. piñnata* (Lam.) O. Oktze*, D. kirtii* Mast, *R. densifolia and V. brachycalyx Haffm* (Table 1).

Phytochemical constituents

The different solvents used for the extractions were seen to extract similar or different compounds from the various plants (Table 1). The key medicinal plants tested were found to contain various compounds which may or may not contribute to their activities. From the study almost all the plants extracts gave positive test for steroids and terpenoids and none of them tested positive for flavanoids and Emodols (Table 1). Alkaloids were detected only in the methanol extracts of *S. pinnata* extracts of *C. myricoides* R.B. and S. *areliacea* Hosct*,* but only in the methanol and water extracts of *T. emetica* Vahl, in the methanol extracts of *S. pinnata* (Lam). O. Oktze, and ether extracts of *D. kirkii* Mast. Emodol(Lam). O. Oktze*.* Courmarins were detected in all the was detected only in the ether extracts of *R. densifolia.* Flavanoides was not detected in any of the extracts tested (Table 1).

Bioactivity test

The extracts of the plants species (Table 2) showed variations in their ED_{50}'s hence the level of toxicity to the brine shrimp larvae (Table 3). All the extracts for *D. Kirtii* Mast and *R. densifolia* were found inactive at both very low and high concentration (0.3 µg/ml). Water extracts of *H. gerberifolium* Sch. BIP and that of *S. pinnata* (Lam). O. Oktze, were found to be inactive at their highest concentrations of 4.0 and 0.30 µg/ml repectively Methanol extracts of *C. myrcoides* R.B. for all the concentrations used killed all the nauplii at the lowest concentration of 0.10 µg/ml. W µg/ml extracts of *G. psittacinus* L. also killed all the nauplii at the lowest concentrations of 0.10 µg/ml.

DISCUSSION

In this study, chemical quantitative analysis was carried out on the extracts of eleven key plant species (Table 1). In the ether extracts, all the plant species analyzed, steroids and terpenoids were detected. Reducing compounds were detected in the methanol extracts of all the species except in *H. gerberifolium* Sch. BIP. and *S. pinnata* (Lam). O. Oktze. Alkaloids were detected only in the methanol extracts of *S. pinnata* (Lam). O. Oktze. Caumarins was detected in all the three extracts of *C. myricoide* and *S. arliacea* but only in the methanol and water extracts of *T. emetica*, in the methanol extracts of *S. pinnata* and ether extracts of *D. kirtii.* Saponins were detected in water extracts of the *P. kotschy*, in the methanol and water extracts of *G. psittcinus* L. and only in the methanol extract of *T. emetica*, *S. pinnata* (Lam). O. Oktze, *R. densifolia* and that of *V. brachycalyx* and only in the water extracts of *R. densifolia* and that of *V. brachycalyx*. Emodols was detected only in the ether extracts of *R. densifolia*. Tannins were detected in the methanol and water extracts of *P. kotschy*, *C. myricoides, T. emetica, S. pinnata* (Lam). O. Oktze, *R. densifolia* and *H. gerberifolium* Sch. BIP. But only in the water extracts of *S. areliacea* (Table 1). According to the study, all the plant species extracted contained one or more chemical compounds with one or more solvents used. This indicates that, all plants have chemical compounds which may or may not contribute to their bioactive characteristics.

Table 2. Showing the results of Brine shrimp lethality test at 200 µ/ml of extracts solution.

Species	Solvents	ED_{50}	Upper limit	Lower limit	Chi-square calculated	Chi-square table	d.f
	Ether	0.3113	0.3795	0.2472	25.8392	7.82	3
H. gerberiforlium Sch. BIP	Methanol	0.2324	0.2907	0.1799	4.1393	5.99	2
	Water	Inact	Inact	Inact	Inact	inact	Inact
	Ether	0.6421	0.7612	0.5341	25.5319	9.49	4
C. hirsute Perr. and Gull	Methanol	0.2346	0.3235	0.1512	6.2238	7.82	3
	Water	0.6412	0.7763	0.5094	15.4992	5.99	2
	Ether	0.1323	0.1763	0.0845	3.4779	7.82	3
S. areliacea Hosct	Methanol	0.3654	0.4437	0.2939	13.5041	7.82	3
	Water	0.0298	0.0663	0.0050	11.6335	7.82	3
	Ether	1.2984	1.7321	0.9971	9.4063	7.82	3
P. kotctchyi (Sceinf.) Harms	Methanol	0.2765	0.3580	0.1903	0.6346	9.49	4
	Water	Incon	Incon	incon	Incon	7.82	3
	Ether	2.6045	14.8039	1.51330	0.0697	5.99	2
V. brachycalyx Haffm	Methanol	0.2989	0.3580	0.2193	5.0131	7.82	3
	Water	1.4821	1.7951	1.2317	23.0891	9.4	4
	Ether	0.0894	0.1539	0.0298	2.7087	7.82	3
C. myriciodes RB	Methanol	all died	all died	all died	All died	all died	all died
	Water	1.8218	2.4738	0.11960	9.4117	all died	all died
	Ether	0.1244	0.515	0.1021	90.8285	7.82	3
S. pinnata (Lam). O. Oktze	Methanol	0.2939	0.6484	0.2216	0.1979	3.84	1
	Water	Inact	Inact	inact	Inact	inact	Inact
	Ether	1.0758	1.2518	0.9269	11.3707	5.99	2
T. emetica Vahl	Methanol	0.1108	0.1367	0.0803	13.1868	3.84	1
	Water	0.7624	0.8855	0.6330	89.9978	5.99	2
	Ether	0.3172	0.4537	0.1687	1.3052	7.82	3
G. psitticinus L	Methanol	0.1119	0.1496	0.0693	2.5036	5.99	2
	Water	all died	all died	all died	All died	All died	All died

Inact = inactive, ED_{50} (Effective dose 50) is the amount of material required to produce a specified effect in 50% of an animal population by swallowing, skin contact or by injection. The Chi square for each plant species was calculated to test for the null hypothesis and the values read at 95% confidence interval for each plant species.

Table 3. Toxicity of plant extracts to brine shrimp larvae in 200 µ/ml solution of the extracts.

Plant species	Methanol extracts	Ether extracts	Water extracts
H. gerberiforlium	60	58	0
C. hirsute	60	57	60
S. areliacea	60	60	60
P. kotctchyi	58	31	10
V. brachycalyx	57	28	23
C. myriciodes	60	60	28
S. pinnata	10	60	1
T. emetic	60	52	60
G. psitticinus	60	56	58
D. kirtii	0	0	0
R. denifolia	0	0	0

Numbers are maximum values of shrimps that died in extract solution. Maximum amount of volume of extracts used was 200 µl and total number of brine shrimp larvae used was 60 each.

Almost all the plant extracts gave positive test for steroids and terpenoids with just a few exception yet these plants are quite distinct species. None of the plant extract tested positive for flavanoids and emodols. This could be possibly because these were not the right solvents used. There were also some plant compounds which were detected in some plants and not in the others confirming that plants composition are not usually alike hence their differences (Table 1). These compounds should not however, be taken as a confirmatory characteristics for these plants.

Bioactive test

The extracts were then tested for their bioactivities as indicated by their ED_{50}'s. Toxicity to the brine shrimp larvae, showed that some of them are not toxic at all while others had low toxicity or were very toxic. The results showed variations in the ED_{50}'s of all the extracts (Table 2). The ED_{50}'s of some of the extracts were not obtained because they were not toxic at all or were very toxic as they killed almost all the shrimp larvae in all concentrations used (Table 3). The ED_{50}'s for ether extracts of *C. myricoides* R.B. was the lowest followed by that of *S. piñnata* (Lam.) O. Oktze*, S. areliacea* Hosct*, H. gerberifolium* Sch.BIP*, G. psittcinus* L.*, C. hirsute* Perr. and Gull.*,* and others showing the ranges in their level of toxicity from lowest to highest (Table 2). For the methanol extract, the ED_{50}'s for *C. myricoides* R.B. was not obtained because even after repeated test, the extracts killed all the shrimps or almost all the shrimps indicating its high toxicity. However, the methanol extracts of *T. emetic* Vahl had the lowest ED_{50}'s value also showing its high toxicity. This was followed by *G. psittacinus* L.*, H. gerberifolium* Sch.BIP*, C. hirsute* Perr. and Gull*, P. kotschyi* (Sceinf.) Harms*, S. pinnata* (Lam.) O and *V. brachycalyx* Hoffm. The ED_{50}'s for the water extracts of *H. gerberifolium* Sch. BIP*,* were not however, obtained because the extract never showed any toxicity against the brine shrimp larvae. Even after 24 h, all the napauli remained active. This means this extract is less toxic even with the lowest concentration, that is at 0.10 µml and highest 2.00 µg/ml (Table 3). The ED_{50} for water extracts of *G. psittacinus* L. was also not obtained because all the shrimp larvae died at all the concentrations even after repeating the tests, the lowest concentrations being 0.25 µg/ml and the highest 3.00 µg/ml. This is an indication that it is very toxic (Table 3). The ED_{50}'s for *P. kotstchyi* (Sceinf.) Harms were not obtained because the data were inconsistent after repeating the tests (Table 2). The water extracts of *S. areliacea* Hosct showed high toxicity as observed from its low ED_{50}'s. This was followed by that of *C. hirtsute* Perr. and Gull, *T. ematica* Vahl. and *V. brachycalyx* Hoffm.*,* respectively. The napauli died in the trend of the level of toxicity (Table 2).

Within the extract of particular species, variations we re also observed in their ED_{50}'s. The methanol extracts of *H. gerberifolium* Sch.BIP showed the lowest ED_{50}'s compared to the ether and water extracts indicating that it is more toxic. For *C. hirsute* Perr. and Gull, the methanol extracts also showed the lowest ED_{50}'s followed by water and ether extracts. This means that the methanol extracts are still more toxic followed by that of ether and then finally water. For those of *S. arealiacae* Hosct the water extracts showed a very low ED_{50} showing that it is very toxic. The ether extract was then seen to have a smaller ED_{50} compared to that of Methanol. This therefore means that it is more toxic than methanol extracts. For *P. kotstchyi* (Sceinf.) Harms*,* the methanol extract with a lower ED_{50}'s is seen to be more toxic than the ether extracts. Methanol extracts for *V. brachycalyx* Hoffm. was more toxic than the water and the ether extracts though the water extracts was also more toxic than the ether extracts.

The ether extracts for *C. myricoides* Perr. and Gull are low hence they are more toxic than the water extracts. Among the extracts of *T. emetic* Vahl, the ED_{50}'s for the methanol extracts is lowest hence toxic. The water extracts also shows more toxicity than that of ether due to its lower ED_{50}. All the extracts of *G. psittacinus* L. showed high toxicity but the water extracts was more toxic than the others because almost all the shrimp larvae died at all concentration used even when the tests were repeated. The methanol extracts for this plant also showed high toxicity than the ether extracts as seen from the differences of their ED_{50}'s. All the three extracts of *D. kirkii* Mast, and those of *R. densifolia* showed no toxicity. No brine shrimp larvae were seen dead even after repeating the tests for the various concentrations used.
The water extracts of *S. pinnata* (Lam.) O. Oktze was seen to be in active even at the highest concentration at 0.30 µg/ml. All in all the methanol extracts for all the plants species were more toxic than all the other extract.

Although some of these plants had similar secondary metabolites (Table 1). Their toxicity against the brine shrimp larvae were seen to be varying (Table 2). The reasons for these variations are not quite clear but it could be that some of the active compounds of these plants were not tested. Secondly the extract were not purified/isolated, therefore, there could have been some components which had inhibitory roles in the test experiments.

According to Olwa Odyek (Pers. Com), the brine shrimp test is not enough to disqualify the medicinal values of these plants variation could be a result of other factors like temperature variations during the process which might denatured some of the active compound in the plant. He said the quantitative analysis only gives a rough guide about what the extracts contain. He elaborated that some of the compounds are in such tiny amount that cannot be detected by this method and this might also contribute to the toxicity of the plants. He also said that these plants may be active only when fresh.

Conclusion

The study shows that the various plant species that exist in Erute county may or may not have similar plant components which contribute to their medicinal value. Although some of these plants had similar secondary metabolites, their toxicity against the brine shrimp larvae was varying. The reasons for these variations are not quite clear but it could be that, some of the active compounds of these plants were not tested and the right solvents used. Secondly the extract were not purified/ isolated, therefore, there could have been some components which had inhibitory roles in the test experiments. Some of the components might also be in very little amounts that cannot be detected by these methods and these might also contribute to the toxicity of the plants. Most of the inactive extracts were seen to contain steroids / trepenoids and the most active or toxic ones contained saponins. These components may therefore be the ones contributing to their varying ranges of toxicity. Also some of the plants had little or no toxicity at all, yet those were the plants that most of the healers were using for the treatment of various diseases. This, to the researcher's opinion, this could serve to disapprove their claim and hence the need for further evaluation/ investigation of these plant resources.

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