Journal of Animal & Plant Sciences, 2011. Vol. 11, Issue 1: 1364-1373
Publication date: 30/8/2011, http://www.biosciences.elewa.org/JAPS; **ISSN 2071 - 7024**

Phytochemical and cytotoxicity testing of *Indigofera lupatana* Baker F.

*[1] Ngoci S. N., [1]Mwendia C. M and [1]Mwaniki C. G.

[1]*Dept. of Biochemistry and Molecular Biology, Egerton University, Box 536, Egerton, Kenya.*
*Corresponding author: hicogn@gmail.com
Key words: Phytochemicals, Cytotoxicity, Brine shrimp, *Indigofera lupatana* Baker. F.

1 SUMMARY

Indigenous plants are promising as a cheap and alternative complimentary medicine (CAM). Although the use of phytomedicine has been in practice for long, little has been done to evaluate their effectiveness, safety, target organisms and also their chemical characterization. *Indigofera lupatana* Baker F. (locally known as *Mugiti*) has been used by Mbeere community of Kenya to treat many conditions such as cough, diarrhea, pleurisy and gonorrhea. In this study, powdered sample of *I. lupatana* Baker F. roots were sequentially extracted using hexane, ethyl acetate, dichloromethane and methanol solvents. The resultant extract fractions were subjected to phytochemical tests and brine shrimp cytotoxicity bioassay. Results showed that the fractions had Phenolics, Flavonoids, Tannins, Saponins, Terpenoids, Cardiac glycosides, Steroids and Phlobatannins. These are responsible for the bioactivity of the sample fractions. All fractions had a LC_{50} value greater than 1000µg/ml which is an indication that they are all non toxic. Therefore *Indigofera lupatana* Baker F. can be used as an alternative source of new, effective, safe drug(s) because it demonstrated presence of phytochemicals with no oral toxicity.

2 BACKGROUND INFORMATION

Medicinal plants contribute significantly to rural livelihoods. Apart from the traditional healers practicing herbal medicine, many people are involved in collecting and trading medicinal plants. The result is an increased demand worldwide leading to enhanced new drugs. The World Health Organization (WHO) estimates that 80% of the world's population depends on medicinal plants for their primary health care (Gurib-Fakim and Schmelzer, 2007; Mothana *et al*., 2008).

Natural products are an important source of new anti-microbial agents. Drugs derived from unmodified natural products or semi-synthetic drugs obtained from natural sources accounted for 78% of the new drugs approved by the United States Food and Drug Administration (FDA) between 1983 and 1994 (Suffredini *et al*., 2006). This evidence contributes to the support and quantification of the importance of screening natural products.

Studies aimed at finding and characterization of the substances that exhibit activity against infectious micro-organisms, yet showing no cross resistance with existing antibiotics, are urgently required (Olila *et al*., 2001). In recent years, pharmaceutical companies have focused on developing drugs from natural products. Also, discovery of new drugs has been made a continuous process to counter the limitations of conventional antibiotics (Doughart and Okafor, 2007), and hence the driving force of this study.

Medicinal plants form the largest single group of plants (Gurib-Fakim and Schmelzer, 2007). *Indigofera lupatana* Baker F. locally called 'mugiti' is a woody shrub found in Acacia-Combretum ecological zones of Mbeere District in Kenya. It is widely used for its perceived medicinal value in treating coughs and diarrhea (Riley and Brokensha, 1988), gonorrhea and pleurisy (Kokwaro, 1993). There is apparently no documented scientific report on phytochemical studies and toxicity profiles of this plant. This has often constituted a major constraint to consideration of the use of herbal remedies in conjunction with or as an affordable alternative to conventional medical treatment (Okeke *et al*., 2001). The bio-activity of natural products is due to phytochemicals, often elaborated for the plant defense. These phytochemicals inadvertently protect humans against pathogens. Some phytochemicals are known to have therapeutic and prophylactic properties, provide nutrition for normal cell health and repairs, inhibit carcinogens and act as antioxidants. The phytochemical screening of plant materials to determine the presence of bioactive chemical constituents is thus vital in the knowledge of their therapeutic properties (Ogunwenmo *et al*., 2007). This can be done chemically, or through qualitative thin layer chromatography (TLC).

Knowledge of the chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in disclosing new sources of such economic substances such as; tannins, oils, gums, and precursors for the synthesis of complex chemical substances. In addition, the knowledge of the chemical constituents of plants would further be valuable in discovering the actual value of folkloric remedies (Mojab *et al*., 2003).

Despite the beneficial effects of phytochemicals, studies have established that they can be toxic (Orech *et al*., 2005). These chemicals are produced as part of the plant's defense against pests and herbivores or to gain an advantage over competing agents. Therefore, medicinal plants are not always safe (Orech *et al*., 2005). It is therefore appropriate to evaluate the potential cytotoxicity of the plant extracts (said to indicate useful antineoplastic activity). This will be used to create awareness of the safety of the plant extract(s) as a medicine (Houghton and Raman, 1998; Orech *et al*., 2005; Suffredini *et al*., 2006). Therefore the objective of this study was to evaluate the cytotoxicity and phytochemical profiles of *I. lupatana* Baker herbal plant.

3 MATERIALS AND METHODS

3.1 Collection and Identification of Plant samples: The plant samples for the study were collected from Mbeere district, in Eastern province, Kenya. The plant was taxonomically authenticated at the Department of Biological Sciences of Egerton University. A voucher sample was assigned a reference number (NSN1) and banked in the department herbarium

3.2 Plant root preparation and extraction: The plant roots were separated, washed, cut into small pieces, air-dried in dark at room temperature to a constant weight and ground into a powder by a mill (Thomas-Wiley laboratory mill, model 4). The powder was extracted by sequential process of four organic solvents of increasing polarity; Hexane, Ethyl acetate, Dichloromethane, and Methanol in that order (Houghton and Raman, 1998; Wojcikowski *et al.,* 2008).

3.3 Phytochemical Tests: Phytochemical tests were done to determine the class of compounds in the active fractions. These were identified by characteristic colour changes using standard procedures according to Houghton and Raman, (1998), Edeoga *et al*., (2005) and Shanmugavalli *et al.,* (2009), and the results were reported as (+ve) for presence, and (-ve) for absence. They included the following tests:

3.3.1 Alkaloids: A portion of extract (0.2 g) was dissolved in 1 ml of 1% sulphuric acid. To the acid solution, a drop of Mayer's reagent was added and a white to buff precipitate would show presence of alkaloids. (Houghton and Raman, 1998; Shanmugavalli *et al.,* 2009).

3.3.2 Saponins: About 0.2 g of powdered sample extract was boiled in 2 ml of distilled water on a water bath and filtered. A fraction of aqueous filtrate measuring 1 ml was mixed with 2 ml of distilled water and shaken vigorously to form a stable persistent froth. The frothing was mixed with about three drops of olive oil and shaken vigorously. Formation of an emulsion confirmed presence of saponins (Edeoga *et al.,* 2005).

3.3.3 Tannins: About 0.2 g of the dried powdered samples were boiled in 10 ml of distilled water in a test tube and then filtered. Positive tests were confirmed by addition of 0.1% $FeCl_3$ solution, resulting in a characteristic blue, blue-black, green or blue-green color (Edeoga *et al.,* 2005).

3.3.4 Test for phlobatannins: About 2 g of powdered sample was boiled with 1% aqueous hydrochloric acid for 5 minutes. A positive test result was confirmed by deposition of a red precipitate (Edeoga *et al.,* 2005).

3.3.5 Test for flavonoids: About 5 ml of dilute aqueous ammonia solution was added to a portion of the aqueous filtrate of the plant extract, followed by addition of concentrated sulphuric acid. A positive test result was confirmed by the formation of a yellow coloration that disappeared instantly (Edeoga *et al.,* 2005).

3.3.6 Test for steroids: About 2 ml each of acetic anhydride and sulphuric acid was added to about 0.5 g of solvent extract. A positive test result was confirmed by change of colour from violet to blue or green (Edeoga *et al.,* 2005; Shanmugavalli *et al.,* 2009).

3.3.7 Test for terpenoids (Salkowski test): About 5 ml of the extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2SO_4 was added to form a layer. A positive test result was confirmed by presence of a reddish brown colouration at the interface (Edeoga *et al.,* 2005).

3.3.8 Test for cardiac glycosides (Keller-Killiani test): About 5 ml of the extract was mixed with 2 ml of glacial acetic acid containing one drop ferric chloride solution. To this, 1 ml of concentrated sulphuric acid was slowly underlayed to the sample mixture. A positive test result was confirmed by the presence of a brown ring at the interface (Edeoga *et al.,* 2005).

3.4 Chemical group tests: The fractions were tested for some of the functional groups as follows:

3.4.1 Phenolic group test: To test for the presence of phenolic groups, 3 to 5 drops of 1M $NaOH_{(aq)}$ were added to 2 ml of the sample. Solubility of the sample was an indication of presence of phenolic groups (Pavia, 1990).

3.4.2 Carboxylic acid group test: To test for the presence of carboxylic acid groups, 3-5 drops of 1M $NaHCO_{3\ (aq)}$ were added to 2 ml of sample extract. Solubility and effervescence of the sample was a confirmation of a presence of carboxylic groups (Pavia, 1990).

3.4.3 Lucas test for alcohol groups: To test for the presence of alcohol/hydroxyl groups, 3-5 drops of Lucas reagent were added to 2 ml of the sample. Formation of green precipitate was a confirmation of positive results. If the reaction took place very fast, tertiary alcohols were present, if moderate reaction took place, secondary alcohol were present and where reaction was unobservable, it indicated presence of primary alcohols (Jerry *et al.,* 1998).

3.4.4 Potassium permanganate test for unsaturation or hydroxyl group: To test for the presence of double and/or triple bonds or OH groups, 3-5 drops of 1 M Potassium permanganate was added drop wise and shaken. Decolourization of potassium permanganate was a confirmation of a positive test (Furniss *et al.,* 1989).

3.4.5 Tollen's test for aldehyde and/or ketone groups: To test for the presence of aldehydes and/or ketones, 3-5 drops of Tollen's reagent were added to 2 ml of the sample. Presence of silver or a black precipitate was a confirmation of a positive test (Jerry *et al.,* 1998).

3.5 Brine shrimp toxicity bioassay: The brine shrimp (*Artemia salina* leach) toxicity bioassay was conducted according to Orech *et al.,* (2005) and Ayo *et al.,* (2007) with few modifications.

Artificial seawater was prepared by dissolving sea salt (38.0 g) in distilled water (1 L). The seawater was put in a small tank and a teaspoon of brine shrimp eggs was added to one side of the divided tank, which was covered. The other side was not covered so as to allow light that attracted the hatched shrimps. The tank containing the brine shrimp eggs was left at room temperature for 48 hours to allow the eggs to hatch.

Different concentrations of plant extract fractions were prepared, using 1% aqueous DMSO. This involved dissolving sample extract (30 mg) in DMSO (3 ml). From this solution, concentrations (1000, 500, 250, 125, and 62.5 μg/ml) were obtained respectively by serial dilution. An aliquot of each concentration (1 ml) was transferred, in triplicates, into clean sterile universal vials with pipette and artificial sea water (9 ml) was added.

Ten shrimp nauplii were added to each vial (30 shrimps per concentration). DMSO aqueous solution (1%) was used as the negative control. All test tubes were incubated at room temperature for 24 hrs. After this period, the number of the dead and the surviving brine shrimps was recorded, and percentage death at each concentration was determined. The LC_{50} value at 95% confidence interval was determined from the counts using the statistical method of probit analysis. The criterion for toxicity for fractions was established as LC_{50} value > 1000 μg/ml (non toxic), ≥500≤1000 μg/ml (weak toxicity) and <500 μg/ml (toxic) (Orech *et al.,* 2005; Ayo *et al.,* 2007; Mbwambo *et al.,* 2007; Bastos *et al.*, 2009).

3.6 Statistical analysis: The determination of LC_{50} value at 95% confidence interval was determined from the count by Probit Analysis using the EPA computer probit analysis program (Version 1.5) (Orech *et al.,* 2005; Mbwambo *et al.,* 2007).

4.0 RESULTS

4.1 Phytochemical results for Indigofera. lupatana Baker F. roots extracts: The phytochemical screening of the extracts of *Indigofera lupatana* Baker F. revealed that alkaloids were absent in all extract fractions while phytosteroids were present in all sample fractions (Table 1). Flavonoids, saponins cardiac glycosides and terpenoids were absent in hexane extracts but all were present in ethyl acetate, dichloromethane and methanol extract fractions. Tannins were present in the more polar extracts of methanol and dichloromethane but absent in the ethyl acetate and non-polar hexane fraction. Phlobatannins were present in the hexane, ethyl acetate and methanol fractions, but absent in the dichloromethane extract fraction.

Table 1: Phytochemical tests results.

Phytochemical constituent	Hexane extract	Ethyl acetate extract	Dichloromethane extract	Methanol extract
Alkaloids	-ve	-ve	-ve	-ve
Flavonoids	-ve	+ve	+ve	+ve
Tannins	-ve	-ve	+ve	+ve
Saponins	-ve	+ve	+ve	+ve
Cardiac glycosides	-ve	+ve	+ve	+ve
Phlobatannins	+ve	+ve	-ve	+ve
Phytosteroids	+ve	+ve	+ve	+ve
Terpenoids	-ve	+ve	+ve	+ve

(+ve) - Represent presence of the tested phytochemicals in the sample fraction
(−ve) - Represent absence of the tested phytochemicals in the sample fraction

4.2 The Chemical results for *Indigofera lupatana* Baker F. roots extracts: The chemical tests revealed presence of phenolic groups, carboxyl groups and hydroxyl groups in ethyl acetate, dichloromethane and methanol extract fractions, but their absence in the hexane fraction. Aldehyde and/or ketone compounds were however present in all extract fractions while unsaturated compounds were present in all fractions except dichloromethane extract fraction.

Table 2: Chemical tests result.

Chemical constituent	Hexane extract	Ethyl acetate extract	Dichloromethane extract	Methanol extract
NaOH test for Phenolic Group	-ve	+ve	+ve	+ve
$NaHCO_3$ test for Carboxyl Group	-ve	+ve	+ve	+ve
Lucas Test for Hydroxyl Groups	-ve	+ve	+ve	+ve
$KMnO_4$ Test for unsaturation	+ve	+ve	-ve	+ve
Tollen's Test for Aldehyde and/or Ketone Groups	+ve	+ve	+ve	+ve

(+ve) - Represent presence of the tested chemical groups in the sample fraction
(−ve) - Represent absence of the tested chemical groups in the sample fraction

4.3 Cytotoxicity test result for the Root Extracts of *Indigofera lupatana* Baker F.: The brine shrimp lethality bioassay results were determined by probit software. From these analyses, it was established that all extract fractions had a LC50 value of greater than 1000μg/ml and hence none was toxic (Table 3).

Table 3: Cytotoxicity test results.

Extract fraction	LC50 (μg/ml)	95% CI	Slope (± SE)	χ^2
Hexane	1370.78	974.76 – 3514.65	2.88 ± 0.80	1.05
Ethyl acetate	2335.15	2026.33 – 2737.86	4.97 ± 0.66	4.61
Dichloromethane	1858.39	1589.06 – 2221.05	3.69 ± 0.47	4.27
Methanol	1248.09	1087.95 – 1457.43	3.87 ± 0.46	3.70

LC50- means the concentration that kills 50% of the population (in μg/ml); 95% confidence interval gives the fiducial limit; 1% DMSO solution was used as negative control and caused no mortality to brine shrimps; CI – Represent confidence interval.

5.0 DISCUSSION:

Arrays of phytochemicals were detected in the sample fractions. These phytochemicals included: flavonoids, tannins, saponins, cardiac glycosides, steroids, phlobatannins and terpenoids. They are normally produced by plants as an evolutionary adaptation to harsh environment or in response to attack by other organisms (Ogunwenmo *et al.*, 2007). They however have been found to inadvertently confer anti-microbial protections to humans due to compounds synthesized in the secondary metabolism (Samy and Gopalakrishnakone, 2008) as well as being immuno-modulative (Okuda2005; Al-Bayati and Al-Mola, 2008).

Tannins were detected in dichloromethane and methanol extracts only. They have physiological role by acting as antioxidants through free radical scavenging activity, chelation of transition metals, inhibition of pro-oxidative enzymes and lipid peroxidation (Navarro *et al.*, 2003; Vit *et al.*, 2008), hence modulating oxidative stress and preventing degenerative diseases. They also inhibit tumor growth by inducing apoptosis (Scalbert *et al.*, 2005) and inhibit mutagenecity of carcinogens (Okuda 2005). They exhibit anti-microbial activity by complexing proteins such as adhesins, substrates, cell wall and cell membrane proteins, hence inactivating microbial adhesion which is the first step in establishment of infections, and also causing cell wall/membrane disruption (Cowan, 1999; Okuda, 2005; Victor *et al.*, 2005; Biradar *et al.*, 2007). They also inactivate microbial enzymes and cell envelope transport proteins by processes that may involve reaction with sulfhydryl groups or through non-specific interaction with the proteins (Samy and Gopalakrishnakone, 2008; Kaur and Arora, 2009).

Phenolics were also detected in ethyl acetate, dichloromethane and methanol extracts fractions.

They function by complexing metal ions (e.g. cobalt, manganese, iron, copper, etc.) necessary for microbial growth as co-factors and activators of enzymes (Okuda, 2005; Biradar *et al*., 2007). They also have anti-viral activity by inhibiting viral reverse transcriptase and inducing DNA fragmentation, and they potentiate host-immune defense (Okuda, 2005; Biradar *et al*., 2007; Ogunwenmo *et al*., 2007). Toxicity to micro-organisms in phenolic compounds depends on the site and the number of hydroxyl groups, with evidence that increased hydroxylation results to increased toxicity (Przybylski *et al*., 1998; Cowan, 1999; Biradar *et al*., 2007; Samy and Gopalakrishnakone, 2008). This was justified further by detection of hydroxyl groups in these extract fractions (Table 2).

Both tannins and phenolics have endocrine role, and they function by interacting with estrogen receptors (Victor *et al*., 2005). They are also anti-inflammatory, molluscicidal and hence important in the control of schistosomiasis (Victor *et al*., 2005). They also have anti-diarrheal, anti-septic, anti-viral, anti-fungal, anti-parasitic, anti-irritant properties, used in curbing hemorrhage, in wound healing, and improving vascular health by suppressing peptides that harden arteries (Victor *et al*., 2005; Awoyinka *et al*., 2007; Ogunwenmo *et al*., 2007).

Flavonoids were also detected in all fractions except hexane fractions. They exert their roles as chain breaking anti-oxidants, and by preventing oxidation of low-density lipoprotein by macrophages and metal ions like copper. This reduces the oxidative stress (Buhler and Miranda, 2000). They also act as 'nature's biological modifiers' as anti-allergens, anti-inflammatory, and induces phase two enzymes that eliminate mutagens and carcinogens (Ogunwenmo *et al*., 2007). They also act as anti-microbial by complexing extracellular and soluble proteins, and bacteria cell wall. More lipophilic flavonoids may also disrupt microbial membranes (Navarro *et al*., 2003; Al-Bayati and Al-Mola, 2008; Samy and Gopalakrishnakone, 2008; Kaur and Arora, 2009). Probable targets on microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane bound enzymes. Still, they may inactivate bacterial toxins (e.g. cholera toxin) and inhibits bacterial glucosyltransferases. Flavonoids are also known to increase coronary flow, to reduce the myocardial oxygen consumption and to lower the arterial pressure (Dong *et al*., 2005). They are also known to reduce capillary fragility (Harborne, 1973), to be anti-trypanocidal (Navarro *et al*., 2003), anti-allergic and also to be anti-spasmodic and hence applied to relief asthma and nose bleeding (Victor *et al*., 2005). Flavonoids lacking hydroxyl groups (-OH) on their structure are more active against the micro-organism than those having -OH, and this supports the idea that their microbial target is the membrane (Cowan, 1999; Samy and Gopalakrishnakone, 2008).

Saponins were also detected in all fractions except hexane fractions. They boost respiratory system as expectorant, and hence activity against cough. This could perhaps justify the already traditionally established function of the plant in the treatment and management of dry coughs. They also have anti-protozoa activity by reacting with cholesterol in the protozoal cell membranes causing cell lyses (Cheeke, 1998). Also, saponins functions as vaccine adjuvant, as anti-inflammatory, emetics, anti-viral, antifungal, insecticidal, molluscicidal, piscidal and as anti-bacterial by inhibiting colonization and boosting the immunity. The mode of action for the anti-bacterial effects may involve membranolytic properties of the saponins as well as lowering of the surface tension of the extracellular medium (Al-Bayati and Al-Mola, 2008). They have antineoplastic activity where they act by reacting with cholesterol rich membranes of cancer cells, and inducing mitotic arrest that causes apoptosis of cell (Sahelian, 2008). This limits cell division and growth. They also bind to primary bile acids, which are metabolized by colon bacteria into secondary bile acids. Some of these are promoters of colon cancer (Cheeke, 1998). Also, saponins increase the blood flow of the coronary arteries, prevent platelet aggregation and decrease the consumption of oxygen by heart muscles (Dong *et al*., 2005). They also have anti-edema, anti-tussive, purgative, anti-hypercholesterol, hypotensive, cardiac depressant and immuno-regulatory properties (Victor *et al*., 2005; Awoyinka *et al*., 2007).

Terpenoids were also detected in all fractions except hexane fraction. They exert their roles as anti-bacteria, anti-amoebic, anti-fungi, anti-viral, anti-protozoan, anti-allergens, as immune boosters and as antineoplasia (Ogunwenmo *et al*., 2007; Roberts, 2007). The mechanism of action is speculated to involve membrane disruption by these lipophilic compounds (Cowan, 1999; Ogunwenmo *et al*., 2007; Samy and Gopalakrishnakone, 2008). This may involve perturbation of the lipid fraction of bacterial plasma membranes, altering membrane permeability hence causing leakage of intracellular materials. This is related to physicochemical characteristics of the active principle such as lipophilicity and water solubility, lipid composition and net surface charge of the bacterial membranes. These phytochemicals can cross the cell membranes, penetrating the interior of the cell and interacting with intracellular targets critical for antibacterial activity (Trombetta *et al*., 2005). They are also used to alleviate epilepsy, to relieve cold, influenza, cough and acute bronchial disease (Victor *et al*., 2005), and this could offer a justification why the plant is used in managing cough.

Phytosteroids and cardiac glycosides were also detected in plant extract fractions. Phytosteroids are used to treat venereal diseases, used in pregnancy to ensure an easy delivery and hormonal balance as well as to promote fertility in women and libido in men. They also act as starting material in the synthesis of sex hormones (Edeoga *et al*., 2005; Victor *et al*., 2005) and hence they are potential source of contraceptives. They are also anti-microbial, analgesic, anti-inflammatory, and immuno-suppressive by inhibiting macrophage activation, blocking the production of pro-inflammatory cytokines. They are also active in managing stomach ailments and in decreasing serum cholesterol levels (Soares *et al*., 2005).

Cardiac glycosides are used in treatment of congestive heart failure, whereby they inhibit $Na+/K+$-ATPase pump that causes positive ionotropic effects and electrophysiological changes. This strengthens heart muscle and the power of systolic concentration against congestive heart failure (Ogunwenmo *et al*., 2007). They are also used in treatment of atrial fibrillation, flatter, and they acts as emetics and as diuretics (Harborne, 1973; Desai, 2000; Awoyinka *et al*., 2007).

Phlobatannins were also found to be present in all extracts fractions except dichloromethane fraction, and their presence suggests the diuretic property (Awoyinka *et al*., 2007) of the plant.

However alkaloids were absent in all the extract fractions, and this could offer justification for the non-toxicity of the plant extracts, since most of alkaloids are associated with toxicity (Sneden, 2005; Victor *et al*., 2005; Ogunwenmo *et al*., 2007).

The brine shrimp (Artemia salina leach) are used in the laboratory bioassay of toxicity through estimation of medium lethal concentration (LC50) (Lieberman, 1999; Ayo *et al*., 2007). Several studies have established Brine Shrimp Test as an excellent benchtop, simple bioassay for the preliminary investigations in discovery, purification, isolation and research of natural products (Lieberman, 1999). The technique is a low-cost test, easily mastered bioassay, utilizing small amount of the test material (Bastos *et al*., 2009). It has been used for preliminary assessment of anti-bacterial, cytotoxicity, pesticidal, antineoplastic and insecticidal activity (Suffredini *et al*., 2006). Other investigators have used the technique for detecting fungal toxins, cynobacteria toxins, heavy metals, food additives, cytotoxic testing of dental materials, home cleaning products and pharmaceuticals (Carballo *et al*., 2002; Lieberman, 1999). Furthermore, studies have showed that there is a positive correlation between the lethality to brine shrimp and corresponding oral lethal dose. Therefore the bioassay present a useful alternative model for predicting the oral acute toxicity of plant extract as well as a model for bioassay-guided fractionation of active cytotoxic and antitumor agent (Parra *et al*., 2001; Ayo *et al*., 2007; Bastos *et al*., 2009).

According to Parra *et al*. (2001), Navarro *et al*. (2003) and Bastos *et al*. (2009), LC50 values less than 1000μg/ml is considered toxic. All tested extract fractions had a LC50 value greater than 1000μg/ml (Table 3) and therefore all the plant extracts were not toxic.

This finding is important in support of the use of this plant for alternative medication. Although members of Mbeere communities have used this plant for centuries without raising any issue of its toxicity, this experiment provide a scientific justification on the safety of the bioactive compounds in this plant.

6.0 CONCLUSION

Phytochemical testing showed that the extracts are rich in tannins, saponins, terpenoids, flavonoids, phenolics, phlobatannins, phytosteroids and cardiac glycosides. These compounds are often associated with the anti-microbial activities of plant extracts, and therefore reflect a potential for the development of novel chemotherapeutic agents or templates which in future may serve as leads for the production of synthetically improved therapeutic agents. Brine shrimp lethality test showed that all the extract fractions were non-toxic justifying the safety of the plant against oral toxicity.

This validated information should be useful to traditional healers and patients on judicious use of the *Indigofera lupatana* Baker F. plant, as its safety can to some extent be guaranteed. The millenarian use of these plants in folk medicine suggests that they represent an economical and safe alternative to treat multi drug resistant, emerging and re-emerging infectious diseases.

7 ACKNOWLEDGEMENT

We are grateful to the department of Biochemistry and Molecular Biology, Chemistry and Biological sciences Egerton University for material support.

8 REFERENCES

Al-Bayati, F. A and Al-Mola, H. F. (2008). Antibacterial and antifungal activities of different parts of Tribulus terrestris L. growing in Iraq. Journal of Zhejiang University Science, 9:154–159.

Awoyinka, O. A., Balogun, I. O. and Ogunnowo, A. A. (2007). Phytochemical screening and in-vitro bioactivity of Cnidoscolus aconitifolius (Euphorbiaceae). Journal of Medicinal Plants Research, 1:063-065.

Ayo, R. G., Amupitan, J. O. and Zhao, Y. (2007). Cytotoxicity and anti-microbial studies of 1, 6, 8-trihydroxy-3-methyl-anthraquinone (Emodin) isolated from the leaves of Cassia nigricans Vahl. African Journal of Biotechnology, 6:1276-1279.

Bastos, M. L. A., Lima, M. R. F., Conserva, L. M., Andrade, V. S., Rocha, E. M. M. and Lemos, R. P. L (2009). Studies on the antimicrobial activity and brine shrimp toxicity of Zeyheria tuberculosa (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. Journal of Evidence-based Complementary and Alternative Medicine, 8:16-21.

Biradar, Y. S., Jagatap, S., Khandelwal, K. R. and Singhania, S. S. (2007). Exploring of Antimicrobial Activity of Triphala Mashi—an Ayurvedic Formulation. Journal of Evidence-based Complementary and Alternative Medicine, 5:107–113.

Buhler, D. R. and Miranda, C. (2000). Antioxidant activities of Flavonoids, Department of Environmental and Molecular Toxicology, Oregon State University. Available from: http://www.Ipi.oregonstate.edu/infocentre/phytochemicals/flavonoids/index.html. - accessed on 17/06/2010.

Carballo, J. L., Hernández-Inda, Z. L., Pérez, P. and García-Grávalos, M. D. (2002). A comparison between two brine shrimp assays to detect in vitrocytotoxicity in marine natural products. Journal of BioMed Central: Complimentary and Alternative Medicine, 2:17-21

Cheeke, P. R. (1998). Saponins: Surprising benefits of desert plants. Available from: http://www.Ipi.oregonstate.edu/sp-su98/htm/. - accessed on 17/06/2010.

Cowan, M. M. (1999) Plant products as anti-microbial agents. Clinical Microbiology Reviews, 12:564-582.

Desai, U. R. (2000). Cardiac glycosides. Available from: http://www.people.vcu.edu/~urdesai/car.htm. - accessed on 17/06/2010.

Dong, T. T. X., Zhao, K. J., Huang, W. Z. , Leung, K. W. and Tsim, K. W. K. (2005). Orthogonal Array Design in Optimizing the Extraction Efficiency of Active Constituents from Roots of Panax notoginseng. Journal of Phytotherapy research, 19:684–688

Doughart, J. H. and Okafor, B. (2007). Anti-microbial activity of Senna alata linn. East and Central Africa Journal of Pharmaceutical sciences, 10:17-21.

Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology, 4:685-688.

EPA Probit Analysis, Version 1.5. Available from: http://www.epa.gov/nerleerd/stat2.htm. - accessed on 25/03/2010.

Furniss, B. B., Hannaford, A. J., Smith, P. W. G. and Tatchell, A. R. (1989). Vogel's Text book of Practical Organic Chemistry. 5th edition, pp. 1226.

Gurib-Fakim, A. and Schmelzer, G. (2007). Medicinal plants in Africa. PROTA, pp. 6.

Harborne, J. B. (1973). Phytochemical methods. Chapman and Hall, London. Pp.52-114.

Houghton, P. J. and Raman A. (1998). Laboratory Hand book for the Fractionation of Natural Extracts. Chapman and Hall, London, 1st edition, pp. 1-153.

Jerry, R. M., Terence, C. M., Christina, N. H. and Neckers, D. C. (1998). Experimental Organic Chemistry. A balanced approach. W. H. Freeman and Company, pp. 497-549.

Kaur, G. J. and Arora, D. S. (2009). Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi. Journal of BioMed Central: Complimentary and Alternative Medicine, 9:30-41.

Kokwaro, J. O. (1993). Medicinal plants of East Africa. East Africa literature bureau, Nairobi Kenya, pp.149.

Lieberman, M. (1999). A brine shrimp bioassay for measuring toxicity and remediation of chemicals. Journal of Chemical Education, 76:1689-1691.

Mbwambo, Z. H., Moshi, M. J., Masimba, P. J., Kapingu, M. C. and Nondo, R. S. (2007). Anti-microbial and brine shrimp toxicity of extracts of Terminalia brownii roots and stem. Journal of BioMed Central: Complimentary and Alternative Medicine, 7:1472-1477.

Mojab, F., Kamalinejad, M., Ghaderi, N. and Vahidipour, H. R. (2003). Phytochemical Screening of Some Species of Iranian Plants. Iranian Journal of Pharmaceutical Research, 2:77-82.

Mothana, R. A., Abdo, S. A., Hasson, S. and Althawab F. M. (2008). Antimicrobial, Antioxidant and Cytotoxic Activities and Phytochemical Screening of Some Yemeni Medicinal Plants. Journal of Evidence-based Complimentary and alternative medicine, doi:10.1093/ecam/nen004:

Navarro, M. C., Montilla, M. P., Cabo, M. M., Galisteo, M., Caceres A., Morales, C. and Berger, I. (2003). Antibaterial, antiprotozoal and antioxidant activity of five plants used in izabal for infectious diseases. Journal of Phytotherapy Research, 17:325-329.

Nguemeving, J. R., Azebaze, A. G. B., Kuete, V., Carly, N. N. E., Beng, V. P., Meyer, M., Blond, A., Bodo, B. and Nkengfack, A. E. (2006). Laurentixanthones A and B, anti-microbial xanthones from Vismia laurentii. Journal of phytochemistry, 67:1341-1346.

Okeke, M. I., Iroegbu, C. U., Eze, E. N., Okoli, A. S. and Esimone, C. O. (2001). Evaluation of extracts of the root of Landolphia owerrience for anti-bacterial activity. Journal of Ethnopharmacology, 78:119-127.

Okuda, T. (2005). Systematics and health effects of chemically distinct tannins in medicinal plants. Journal of Phytochemistry, 66:2012-2031.

Olila, D., Olwa-Odyek and Opuda-Asibo, J. (2001). Anti-bacterial and antifungal activities of extracts of Zanthoxylum chalybeum and Warburgia ugandensis, Ugandan medicinal plants. Journal of African Health Sciences, 1:66-72.

Orech, F. O., Orech, T., Ochora, J., Friis, H. and Aagaard-Hansen, J. (2005). Potential toxicity of some traditional leafy vegetables consumed in Nyang'oma Division, Western Kenya. African Journal of Food and Nutritional Sciences, 5: 1-13.

Parra, L. A., Yhebra, S. R., Sardinas, G. I., Buela, I. L. (2001). Comparative study of the assay of Artemia salina L. and the estimate of the medium lethal dose (LD50 value) in mice, to determine oral acute toxicity of plant extracts. Journal of Phytomedicine, 8:395-400.

Pavia, D. L. (1990). Introduction to Organic Laboratory Techniques. A Microscale Approach. pp. 449-482.

Przybylski, R., Lee, R. Y. and Eskin, N. A. M. (1998). Antioxidant and radical-scavenging activities of buckwheat seed components. Journal of the American Oil Chemists' Society, 75:1595–1601

Riley, B. W. and Brokensha, D. (1988). The Mbeere in Kenya; Botanical identity and use Vol (ii), University press of America, USA, pp. 76-77.

Roberts, S. C., (2007). Production and engineering of terpenoids in plant cell culture. Journal of Nature Chemical Biology, 3:387-395.

Sahelian, R. (2008). Saponins. Available from: http://www.raysahelian.com. - accessed on 24/07/2008.

Samy R.P. and Gopalakrishnakone P. (2008). Review: Therapeutic potential of plants as anti-microbials for drug discovery. Journal of Evidence-Based Complimentary and Alternative medicine:doi:10.1093/ecam/nen036.

Scalbert, A., Johnson, I. T. and Saltmarsh, M. (2005). Polyphenols: Antioxidants and beyond. American Journal of Clinical Nutrition, 81:215-217.

Shanmugavalli, N., Umashankar, V. and Raheem (2009). Antimicrobial activity of Vanilla planifolia. Indian Journal of Science and Technology, 2:37-40.

Sneden, A. S. (2005). Alkaloids. Available from: http://www.people.vcu.edu/~asneden/alkaloids.htm. - accessed on 17/06/2010.

Soares, M. B., Brustolim, D., Santos, L. A., Bellintani, M. C., Paiva, F. P., Ribeiro, Y. M., Tossami, T. C. and Santos, R. (2005). Physalins B, F and G, Seco-steroids purified from Physalis angulata L., inhibit lymphocyte function and allogeneic transplant rejection. Journal of International Immunopharmacology 6:408-414.

Suffredini, B. I., Paciencia, M. L.B., Nepomuceno, D. C., Younes, R. N. and Varella, A. D. (2006). Anti-bacterial and cytotoxic activity of Brazilian plant extracts - Clusiaceae . Memórias do Instituto Oswaldo Cruz, 101:1590-1598.

Trombetta, D., Castelli, F., Sarpietro, M., Venuti, V., Cristani, M., Daniele, C., Saija, A., Mazzanti, G. and Bisignano, G. (2005) Mechanisms of anti-bacterial action of three monoterpenes. Journal of Antimicrobial Agents and Chemotherapy, 49:2474-2478.

Victor, J., Siebert, S., Hoare, D. and Wyk, B. V. (2005). Sekhukhuneland grasslands: A treasure house of biodiversity. Available at; http://www.fao.org/ag/AGP/agpc/doc/show/ SAfrica/sapaper/saessay.htm. - accessed on 17/06/2010

Vit, K., Katerina, K., Zuzana, R., Kamil, K., Daniel, J., Ludek, J. and Lubomir, O. (2008). Mini review: Condensed and hydrolysable tannins as antioxidants influencing the health. Journal of Medicinal Chemistry, 8:436-447.

Wojcikowski, K., Wohlmuth, H., Johnson, D. W., Rolfe, M. and Gobe, G. (2008). An in vitro investigation of herbs traditionally used for kidney and urinary system disorders: Potential therapeutic and toxic effects. Journal of Nephrology, 14:70-79.