INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACY AND CHEMISTRY

Available online at www.ijrpc.com

Research Article

CYTOTOXICITY, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF FIVE PLANT SPECIES USED BY AGRO-PASTORAL COMMUNITIES IN MBULU DISTRICT, TANZANIA

M. Qwarse[1], J. Sempombe[2], MJ. Mihale[1*], L. Henry[3], V. Mugoyela[2] and F. Sung'hwa[4]

[1]Department of Physical Sciences, Open University of Tanzania (OUT), Dar es Salaam, Tanzania, Africa.
[2]Department of Medicinal Chemistry, School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania, Africa.
[3]Department of Science and Laboratory Technology, Dar es Salaam Institute of Technology, Dar es Salaam, Tanzania, Africa.
[4]Department of Chemistry, College of Natural and Applied Sciences, University of Dar es Salaam, Dar es Salaam, Tanzania, Africa.

ABSTRACT

Gymnema sylverstre, Phytolacca dodecandra, Cynoglossum geometrium, Leonotis nepetifolia and Ocimum filamentosum plant species are traditionally used by agro-pastoral communities in Mbulu, Tanzania for treatment of various plant, human and veterinary diseases. Extracts from plants were investigated for antibacterial and antifungal activities using agar well diffusion and broth macro-dilution methods. Extracts were tested in vitro for cytotoxicity using Brine Shrimp Lethality test method. Cytotoxic activities against Brine Shrimps of all plants extracts were greater than that of Potassium permanganate standard. *P. dodecandra* leaf extract showed the highest cytotoxicity with an LC_{50} of 4.57 µg/ml. Roots of *O. filamentosum, P. dodecandra* and *C. geometrium* were potent against brine shrimps with LC_{50} values of 28.08, 34.74 and 71.09 µg/ml, respectively. Antibacterial activities of plants extract ranged between 25% and 75% of gentamycin standard activity with *C. geometrium* exhibiting the highest activity against *Escherichia coli* (ATCC 25922) at minimum inhibitory concentration (MIC) of 37.5 mg/mL. Antifungal activities of plants ranged from 0% to 55% of fluconazole standard activity with P. dodecandra leaves showing the highest activity against *Cryptoccoccus neofomans* (ATCC 66037) at an MIC of 9.4 mg/mL. Phytochemical screening of the plant species indicated the presence of alkaloids, terpenes, tannins, phenols, flavonoids, saponins and glycocides. The observed bioactivities could be associated with presence of these phytochemicals in plant species and lend credence to traditional use of these plant species in curing various plants, human and veterinary diseases.

Keywords: Cytotoxicity, Antibacterial, Antifungal, Minimum inhibition concentration.

1.0 INTRODUCTION

Plants have been locally used in treating different diseases based on their potency as accounted for the presence of the active constituents (Vipra *et al*., 2013). Plants have an in-built system for protection against biotic and abiotic stress conditions due to presence of many phytochemical compounds with biological activities (Darokar *et al*., 1998; Joshi and Sati, 2011; Santi *et al*., 2014). Phytochemicals provide health benefits for humans, animals and plants (Hasler *et al*., 1999; Sexana, 2013). Phytochemical screening of bioactive plants have revealed the presence of phenols, alkaloids, flavonoids, terpenes, saponins, tannins and glycosides which is associated with different biological activities (Ebana *et al*., 1993; Ahmad *et al.,*

2006; Iqbal *et al.,* 2010). The plants containing these phytochemicals serve as alternative, effective, cheap and safe products for treatment of various infections (Vipra *et al*., 2013).

World Health Organization (WHO) estimates that 80% of the population in developing countries depend on the traditional medicine for their primary health care needs, while 85% of people in third world use plants or their products for the health care system (Shome *et al*., 1996; Sheldon *et al*., 1998; Santi *et al*., 2014). The increased use of the plants for treating different diseases have resulted into depletion of these species and have made tremendous promotion of the synthetic industry by using the chemistry of natural products. Currently, fungi and bacteria seem to develop resistance to the antibiotics used for the treatment of animals and human diseases. Worldwide, natural products are a prospective source for new modern medicines and about 40% have been developed. Particularly 60 –80% of antibacterial and anticancer medicines were from natural products. In 2001, eight medicines (amoxicillin, simvastatin, clavulanic acid, pravastatin, azithromycin, cyclosporine, ceftriaxone and paclitaxel) out of the 30 top-selling were of natural products origin or their derivatives (Sarker *et al*.,2006) Some of these medicines have serious undesirable adverse effects which limit their applications in the clinics (Jeyachandran and Mahesh, 2007). Therefore, new medicines are immediately required for effective treatment of emerging infections.

Gymnema sylverstre, Phytolacca dodecandra, Cynoglossum geometrium, Leonotis nepetifolia and *Ocimum filamentosum* are plant species used by agro-pastoral communities in Mbulu district, Tanzania to treat various veterinary, crops and human diseases. For example, *G. sylverstre* roots are used by the Harzabe people in Mbulu district for treatment of gonorrhea and measles. Whereas, *C. geometrium* root tubers are used for the treatment of measles in cattle, leaves of *L. neptifolia* are used for treatment of skin fungal disease in humans. Nonetheless, *P. dodecandra* leaves are used by subsistence farmers in treatment of skin fungal infections (Qwarse, 2015).

To the best of our knowledge, the prevalent use of these plant species in Mbulu district, Tanzania have not yet been scientifically screened against various microbial pathogens and for cytotoxicity. This study aimed at evaluating the biological activities (cytotoxicity, antibacterial and antifungal activities) of *G. sylverstre, P. dodecandra, C. geometrium, L.s nepetifolia* and *O. filamentosum* of selected species commonly used by agro-pastoral communities in Mbulu district, Tanzania. The knowledge obtained will establish a scientific rationale for continued traditional use of the plant species and for developing bioactive products as well as providing new sources of prototypes for synthesis of various bioactive compounds.

2.0 MATERIALS AND METHODS

2.1 Study area

The study was carried out in Mbulu district, south west part of Tanzania (Figure 1) with approximately 7,695 square km (including Lake Eyasi), of which 6,700 square km is a dry land.

(**Fig. 1: Map of Tanzania showing Mbulu District (Modified from Google Earth)**

Mbulu district have an altitude which ranges from 1,110 m to 2,250 m. This difference in altitude contributes to the wide range of climatic conditions with mean annual temperature ranging between 17.3 ^{o}C and 23.4 ^{o}C. In addition, the mean annual rainfall ranges from 400 mm to 1,100 mm. The district is mainly dominated by hunters and agro-pastoral communities. There are other socio-economic activities which are practiced including smallholder rainfed cultivation, extensive grazing, afforestation and mechanised rainfed cultivation with medium to high inputs. The main crops grown in the study area include maize, beans, pigeon pea, sorghum, wheat, vegetable, fruits and coffee (Magoggo *et al*., 1994; Brornsen *et al*., 2006). *P. dodecandra* leaves are used by small farmers to control grain pests in stores and field crops with minimal loss of quantity and quality in Mbulu district (Qwarse, 2015).

2.2 PLANT MATERIALS

The fresh samples of *Gymnema, sylverstre* roots*, Phytolacca dodecandra leaf and roots, Cynoglossum geometrium* roots*, Leonotis nepetifolia* leaves *and Ocimum filamentosum* were collected in Mbulu district in March 2014. The plant species were identified and authenticated by Haji Selemani, (taxonomist) at the Herbarium in the Department of Botany, University of Dar es Salaam where voucher specimens were deposited.

2.3 Sample preparation and extraction

Plant materials were immediately separated into their component parts (leaves and roots), placed in the open containers in a dark room and allowed to air dry at room temperature with low humidity for about two weeks**.** The samples were subjected to size reduction where the roots were sliced into pieces of 1 – 2 cm long, while leaves were cut into pieces of

approximately 1 cm^2. All samples were then dried at room temperature for another two weeks.

Dried samples were grind into fine powders using a laboratory electric hammer mill. The powdered plant materials were then stored in air-tight glass jars in a cool place ready for extraction. Extraction of the samples was carried out using cold method (maceration) where plant powders were extracted using ethanol (95% v/v) for 24 hours at room temperature (Omoregbe *et al*, 1996). Later, the extracts were filtered, concentrated, and then stored in a refrigerator at -4 °C for bioactivity tests.

2.4 Toxicity tests

The Brine Shrimp Test (BST) was conducted according to Meyer *et al.,* (1982) and Zhao *et al.,* (1992) with some little modifications as described by Kidukuli *et al*., (2010). Brine shrimp (*Artemia salina* Leach) larvae were used as an indicator for preliminary cytotoxicity assay of the five plant species extracts. Artificial seawater was prepared by dissolving sea salt (38 g) in distilled water to make a concentration of 38 g/L and then filtered. The salt solution was filled into a tank that has been divided into two unequal compartments by perforated polythene wall. Shrimp eggs were later sprinkled into the covered part of the tank and a lamp was illuminated on the uncovered part in order to attract the hatched shrimps. The mature nauplii brine shrimp were collected in between 36 and 48 hours of hatching (Baraza *et al*., 2007). The selected plant species extracts were dissolved in Dimethyl sulfoxide (DMSO) in vials in triplicate at an initial concentration of 240 µg/ mL and decreasing up to 4 µg/ mL. Then every vial containing the extract in solution, 10 brine shrimp larvae were added. Furthermore, the fourth set of vials containing only a solvent (DMSO) in 5mL of artificial seawater, 10 shrimp larvae were added as a control (Meyer, *et al*., 1982). Finally the number of survived larvae was established after 24 hours and the LC_{50} values were obtained using probit analysis. Percentage death was given by the formula:

Percentage death (%) = $\frac{\text{Total } nauplii - \text{Alive } nauplii}{\text{Total } nauplii}$ X 100%

2.6 Antimicrobial activities

The test organisms; five gram-negative and one gram-positive bacterium were used for antibacterial tests. The gram-negative bacteria used were *Salmonella typhimurium* (ATCC 259239), *Escherichia coli* (ATCC 25922), *Shigella dysentariae (clinically isolated), Pseudomonas aeruginosa* (ATCC 27853), *Klebsiella pneumonia* (ATCC 10031) and a gram-positive bacteria was *Staphylococcus aureus* (ATCC 25923). Microorganisms used for antifungal tests included *Aspergillus niger* (mould) (ATCC 16404), *Candida albicans* (yeast) (ATCC 90028) and *Cryptoccoccus neofomans* (yeast) (ATCC 66037). All microorganisms were obtained at the Microbiology laboratory, Muhimbili University of Health and Allied Sciences (MUHAS) where standard culture whose susceptibility on commonly used antibiotics have been well established. Antibacterial and antifungal activities of ethanol extracts of selected medicinal plants were tested using Agar well diffusion method as described by Barros *et al*., (2007c)

2.6.1 Antibacterial activity test

Bacterial Media (Muller Hinton Agar Media) for antibacterial activity were prepared using standard procedures. Thirty six grams (36g) of Muller Hinton Agar Media (Hi-Media) were mixed with distilled water in $1dm^3$ and then sterilised in autoclave at 15 lb pressure at 121°C for 15 minutes. The sterilized media were poured into Petri dishes and left to solidify. Then, wells of 6 mm diameter were made on the agar surface using corks borer. The bored plates were used for the antibacterial screening.

The prepared culture plates were inoculated with different bacteria by using Agar well diffusion method. The extracts were poured into the wells using sterile syringe. The plates were incubated for 24 hours at 37 ± 2 °C for bacterial activity. Then the plates were observed for the zone formation around the wells. After that, the zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. Finally the readings were taken in three different fixed directions in all 3 replicates and the average values were determined.

2.6.2 Antifungal activity test

Two hundred grams (200 g) of potato slices were boiled with distilled water in 1 dm^3 to prepare potato infusions. Dextrose (20 g) mixed with potato infusion agar (20 g) was added as a solidifying agent. The contents were mixed and autoclaved. Wells of 6 mm diameter were made on the agar surface using corks borer and the bored plates were used for antifungal screening.

The prepared culture plates were inoculated with different fungi by using Agar well diffusion method. The extracts were poured into the

wells using sterile syringe. The plates were incubated for 48 hours at 37 ± 2 °C for fungal activity. The plates were observed for the zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter. The readings were taken in three different fixed directions in all 3 replicates and the average values were determined.

2.6.3 Minimum inhibitory concentration (MIC)

Broth dilution method was used to determine the MIC of extracts against the tested organisms that shows positive activities in the Agar Well Diffusion Method. Ethanol extracts from the five plant species showing antibacterial and antifungal activity were tested further on the same bacterial and fungal species to determine the Minimum Inhibitory Concentrations (MIC). The lowest concentration of an extract tested on an organism and shows no any visible growth was taken as its MIC. This was determined by serial dilution method on ethanolic extracts. Bacterial (Muller Hinton broth) and fungal (Sabourand Dextrose Agar) growth media were prepared at concentration of 38 g/dm^3 and 65 g/dm^3 in distilled water respectively, autoclaved at 15 Ib pressure for about 2 hours and sterilized at 120 °C. The sterilised media were poured into Petri dishes and stored in a refrigerator at - 4^{0}C for MIC analysis. The Petri dishes poured with medium were divided into twelve parts by labelling it at the bottom with 1, 3, 5, 7, 11, and 12 numbers. The incubation and growth of the organisms were done for 24 hours at 37 °C for antibacterial activities and 48 for antifungal activities (National Committee for Clinical Laboratory Standards, 1990, 1997). Stock solutions of plant extracts were prepared by dissolving 500 mg in DMSO (1 mL). Serial dilutions were performed in the 96 well plates by adding 50 µl of media in 12 holes in replicates. Then, the extract (50 µL) was added serially up to the 8th hole. At the 12th hole standard commercial antibiotic gentamycin and antifungal fluconazole were added as positive control for bacterial and fungal tests respectively. DMSO (50 µL) was added in the 9th hole as a negative control and 10th and 11th holes were left untreated. Then, microorganism culture (50 µL) either bacteria or fungi was added to each hole. All 96 well plates were incubated for 24 hours at 37 °C for antibacterial tests and 48 hours for antifungal tests. The lowest concentration at which the tested organism shows no visible growth was taken as its MIC. After every experiment, all experimental materials were sterilized in the autoclave and disposed as required for environmental and health safety regulations.

2.7 Qualitative screening of phytochemicals

Chemical tests were carried out using the ethanol extracts to identify the constituents as described below. The presence of saponins in the plant extracts was determined as described elsewhere (Kokate, 1996). The extract was mixed with distilled water to make 20 mL and the suspension was shaken for 15 min and then form of a 2 cm layer of foam which indicates the presence of saponins.

Tannins and flavonoids, coumarins and anthraquinones were screened according to (Buvaneswari, 2011). To screen tannins, water (20 mL) was added in a test tube containing dried plant powder (0.5 mg) then boiled and filtered. Later, drops of Ferric chloride (0.1 %) were added in a filtrate. The appearance of brownish green or blue black coloration indicates the presence of tannins (Buvaneswari, 2011). To screen flavonoids, diluted ammonia solution (5 mL) was added to a portion of the aqueous extract (6 mL), followed by few drops of concentrated sulphuric acid. Then the appearance of yellow coloration indicates the presence of flavonoids (Buvaneswari, 2011). Coumarins were screened using sodium sulphate. Few drops of Sodium sulphate were added dropwise to a crude extract (5 ml) of ethanol extract in a test tube. Appearance of a yellow colour indicates the presence of coumarins (Buvaneswari, 2011). Similarly, presence of anthraquinones was evaluated using benzene. Few drops of benzene were added dropwise to a crude extract (5 ml of ethanol extract) in a test tube followed by ammonia drop. Appearance of a pink colour indicates the presence of anthraquininones (Buvaneswari, 2011).

Glycosides in the extracts were tested using Liebermann's test as described by (Yadav and Agarwala, 2011). Crude plant extract (6 mL of ethanol extract), was mixed with chloroform (2 mL) and acetic acid (2 mL). The mixture was cooled in ice and then few drops of concentrated H_2SO_4 were added. A colour change from violet to blue and then to green, indicates the presence of steroidal nucleus, i.e., glycone portion of glycoside.

Sterols, triterpenoids and phenols were also screened using a method described by Yadav and Agarwala, (2011). The sample extract (6mL of ethanol extract), 2mL of chloroform and few drops of concentrated H_2SO_4 were added. Later the mixture was shaken and then allowed to stand for some time for the colour to appear .When it appears red colour in the lower layer indicates the presence of sterols,

while the appearance of yellow colour in lower layer indicates the presence of the triterpenoids (Yadav and Agarwala, 2011). To screen phenols, distilled water (5 ml) and few drops of neutral Ferric chloride solution (5%) were added to the plant extract (10 mL of ethanol extract) then dark green colour appears. A dark green colour indicates the presence of phenol compounds (Yadav and Agarwala, 2011).

Alkaloids were evaluated using a method described by Kumar *et al.,* (2011). Chloroform (10 mL) was added into the test tube containing dry plant extract (0.5 g). Then, chloroform evaporates to dryness and the residue dissolved in dilute hydrochloric acid (2 mL). After that, the solution was tested with Dragendorff's reagent and orange precipitate appears. Its appearance indicates the presence of alkaloids (Kumar et al., 2011).

Phlobatannins were evaluated using a method described by Khanna and Kannabiran, (2007). Aqueous extract of plant sample (10 mL of ethanol extract) was boiled with aqueous hydrochloric acid (1%) then red precipitate appears. The appearance of red precipitate indicates the presence of phlobatannins (Khanna and Kannabiran, 2007).

Presence of terpenoids in the extracts was tested using Salkowski test. The plant extract were dissolved in 10 mL of ethanol, and then mixed with 2 mL of chloroform and few drops concentrated sulphuric acid and form layers which were heated for about 2 minutes. A reddish brown or greyish colour interface were formed which signifies the presence of terpenoids (Yadav and Agarwala 2011).

2.8 Data analysis

All experiments were conducted in triplicate and inhibition zone values were expressed as mean ± standard deviation. Data analysis was done using Microsoft office excel 2007. For the MIC values, re-evaluation of the growth inhibition was conducted where variation within the three experiments were notice.

3.0 RESULTS AND DISCUSSION

3.1 Brine shrimp cytotoxicity tests

The results of the brine shrimp lethality bioassay test for the extracts of five plants species are presented in Table 1. Crude ethanolic extracts of *P. dodecandra* leaf extract exhibits the highest LC_{50}. *O. filamentosum* crude extracts had LC_{50} of 28.08µg/ mL. *P. dodecandra* leaves had lower LC_{50} (4.57 µg/mL) compared to other plant species (between 28.08 and 119.42 µg/mL), but higher than that of the Potassium permanganate ($KMnO_4$) standard (LC_{50} =3.39 µg/mL). Moreover, the degree of lethality concentration was directly proportional to the concentration applied, that means mortality increased gradually with the increase in concentration of the tested samples. In general, the LC_{50} extracts of all five plant species were higher than the LC_{50} of the Potassium permanganate ($KMnO_4$) standard.

(Table 1: Cytotoxicity of ethanol crude extracts from five plants species)

Plant toxicity is important in assessing the safety of plant product. The plant's intrinsic toxicity and problem of overdose can be assessed by brine shrimp test, which is considered as a convenient method for preliminary assessment of toxicity due to high sensitivity of the *nauplii* to different chemical substances (Jamil, 2010; Mungenge *et al*., 2014). The numbers of novel antitumor and pesticidal products have been identified and later isolated using this bioassay (Sam, 1993). The LC_{50} values depicted by *P. dodecandra*, *C. geometrium* and *O. filamentosum* extracts indicates the presence of potential cytotoxic compounds in the plants (Peteros and Mylene, 2010; Rahman *et al*., 2014).

The brine shrimp cytotoxicity assay is a convenient method for preliminary assessment of toxicity and has been used for the detection of fungal toxins, food additives, plant extract toxins, heavy metals, cyanobacteria toxins, pesticides, antimicrobial and cytotoxicity testing of dental materials (Rajen *et al*., 2012; Aziz *et al*., 2013; Zimudzi, 2014; Screeshmeal and Nair, 2014). In most cases there are positive correlations reported between brine shrimp lethality and cytotoxiciy toward the human nasopharyngeal carcinoma (9KB cell line), *in vivo* murine leukemia (P388 cell line) and other solid tumors (Sam, 1993). Hence, low LC_{50} is the indicative of the antitumor activity of the plant extract. For example, LC_{50} of *Croton bonplandianum* at concentration of 0.06 mg/mL indicates antitumor properties (Ajoy and Padma, 2013).

3.2 Antimicrobial activities of plants extracts

3.2.1 Antibacterial activities

P. dodecandra was bioactive to *E. coli, S. typhimurium, P. aeruginosa, K. pneumonia* and *S. aureus.* The highest activity as indicates by zone of inhibition was in *S. typhimurium,* followed by *P. aeruginosa* (Table 2). The bioactivity of *P. dodecandra* was 25% to 75% of the activity of gentamycin. This species was not active to *S. dysentriae.* Similarly, *L. nepetifolia* was active to all tested bacterial except *S. typhimurium*. However, the activity of L*. nepetifolia* was lower than that of *P. dodecandra* in all species. The bioactivity of L*. nepetifolia* was 25% to 38% of gentamycin

activity. *C. geometrium* shows bioactivity to *E. coli, S. typhimurium, K. pneumonia* and *S. aureus* only and its bioactivity was 25% to 53% of gentamycin activity. *O. filamentosum* shows activities to four species (*E. coli, S. aureus, K. pneumonia* and *S. dysentry*) and *G. sylverstre shows activities to* three species (*S. typhimurium, P. pneumonia* and *S. aureus*). Bioactivity of *O. filamentosum* was 27% to 48% of gentamycin activity. Similarly, bioactivity of *G. sylverstre* was 31% to 46% of gentamycin activity.

P. dodecandra leaf extract was active against S. *typhimurium*, *P. aeruginosa* and *K. pneumonia* while the root extract was active against *S. typhimurium* and *S. aureus.* These findings could be attributed to the positioning of plant parts on a plant. Plant defence theories suggest that chemical or structural defences should be maximized when and where browsing is most likely to occur (Mathai *et al*., 2000). Leaves are exposed and conspicuous, which makes the best targets for herbivore attack. It is not surprising, therefore, that plants tend to deposit and localize majority of secondary substances in exposed parts such as leaves, barks and fruit/flowers to act as deterrents to herbivores. Plants without conspicuous leaves like *Euphorbia* spp. utilize their green stems/latex for such a purpose (Manu *et al*., 2008). The results obtained from this study show that ethanolic extracts of plant species exhibit antibacterial properties. This justifies their traditional use as medicinal plants.

(Table 2: MIC and Mean Inhibitory Zone of Five Plant Species against Six bacterial Species)

3.2.2 Antifungal activities

P. dodecandra was active against *A. niger, C. albicans and C. neofomans* (Table 2). Leaf extract was active to all fungi except *A. niger,* while root extract was active to all fungi. Bioactivity of *P. dodecandra* leaf extract was 23% to 50% of the fluconazole standard activity. Furthermore, bioactivity of *P. dodecandra* root extract was 23% to 37% of fluconazole activity. The MIC of *P. dodecantra* was as low as that of fluconazole standard for *C. neofomans. L. nepetifolia* and *C. geometrium* extracts. Bioactivity of *L. nepetifolia* was 37% to 53% of fluconazole activity. Similarly, the bioactivity of *C. geometrium* was 23% to 32% of the fluconazole activity. *O. filamentosum* and *G. sylverstre* were both bioactive to *C. albicans* and *C. neofomans* only. Bioactivity of *O. filamentosum* was 26% to 31% of the fluconazole activity. Moreover, bioactivity of *G. sylverstre* was 23% to 25% of the fluconazole activity.

(Table 3: MIC and Mean IZ of Selected Plant Species against Three Fungal Species)

The secondary metabolites in *P. dodecandra* may be responsible for this bioactivity (Mohamed *et al*., 1996). Other studies have shown that chloroform and ethyl acetate extracts of the aerial parts of *G. sylvestre* exhibited activity against *P. vulgaris* (Fabio *et al*., 2013). Traditionally *G. sylvestre* has been used to control pest and disease in India. On the other hand, the ethanol extract of *L. nepetifolia* leaves has been reported to have strong activity against *C. albicans* (Maobe *et al*., 2013). It is known that the environmental factors such as climate, altitude and soil type can influence the diversity of plant species' bioactive compounds (Nalubega 2010; Ngoci, *et al*., 2014). Thus, these investigations have provided more evidence of therapeutic potency of the plant species in traditional medicine.

The roots *of C. geometrium* ethanolic extract demonstrated activity against bacteria (*E. coli, S. aureus, K. Pneumonia* and *S. typhimurium)* and fungi (*C. albicans,* and *C. neofomans*). Omwega and Paul (2012), Chakraborty and Chakraborti, (2010) reported that presence of flavonoids, tannins and cardiac phytochemicals inhibited growth of bacterial and fungal.

3.3 Phytochemical screening of plant species

The phytochemical composition of five plant species is presented in Table 4. The analysis has indicatedthe presence of widespread compounds like saponins, favonoids, alkaloids, sterols and tripenoids. Phenols were present in *Gymnema sylverstre*, *Phytolacca dodecandra*, *Cynoglossum geometrium and Leonotis nepetifolia*. Alkaloids were present in *Gymnema sylverstre*, *Phytolacca dodecandra* leaves*, Ocimum filamentosum and Leonotis nepetifolia.* Terpenes or terpenoids were present in *Gymnema sylverstre*, *Phytolacca dodecandra*, *Ocimum filamentosum and Leonotis nepetifolia.* Tannins were present only in *Ocimum filamentosum and Leonotis nepetifolia.* Sterols and sterol glycocides were present in four plants tested except *Cynoglossum geometrium*. Four bioactive plant extracts tested contains flavonoids in this study except *Gymnema sylverstre.* Saponins were present in all five plants extracts as indicates in Table 4. Anthraquinone and phlobatanins were present only in *L. nepetifolia* (leaf) and *G. sylvestre* (root).

The phytochemical screening of the bioactive plant extracts has revealed the presence of

alkaloids, tannins, flavanoids, sterols, terpenes glycosides, phlobatanis, phenol, saponins and anthraquinone (Table 4). Tannins have been reported to have antibacterial, antifungal and anti-diarrhoeal activities (Ahmad *et al.,* 2006,2014; Nathathe and Ndip, 2011; Ogbonna *et al*., 2013; Ngulde *et al.,* 2013). Secondary metabolites such as saponins, tannins, alkaloids and cardiac glycosides have been reported to treat diseases such as typhoid, haemorrhoids, impetigo and malaria (Okigbo *et al*., 2009; Ogbonna *et al.,* 2013; Adeogum *et al.,* 2014;). Anti-inflammatory effects have also been reported for these phytochemicals including flavonoids (Lui, 2003). Observed activities against bacteria and fungi could be associated with the presence of these phytochemicals in the bioactive extracts.

Tannins may be responsible for antibacterial activity demonstrated by these plant extracts (Table 4). A previous study shows that tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis (Ahmad *et al.,* 2006). Tannic acid which is a mixture of gallic acid esters of glucose can be used as a topical preparation for cold sores (Heinrich *et al*., 2004). Many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumour activity, and a wide range of anti-infective actions, have been associated with the presence of tannins (Haslam, 1996). One of their molecular actions is to complex with proteins through so-called nonspecific forces such as hydrogen bonding and hydrophobic effects, as well as by covalent bond formation (Haslam, 1996; Yadav, and Agarwala 2011). Thus, their mode of antimicrobial action may be related to their ability to inactivate microbial adhesions, enzymes, and cell envelope transport proteins.

(Table 4: Qualitative Analysis of Phytochemical Constituents of Plant Species)

Tannin complex with polysaccharide is an indication of a good antibacterial, antioxidant and/or antifungal activity (Brownlee *et al.,* 1990; Somkuwar and Kamble, 2013). Probably, the observed activity of these plants and their use by subsistence farmers in diseases management is due to the presence of phytochemicals properties.

Presence of alkaloids in the plant species may be responsible for the observed antibacterial and antifungal activity (Table 4). Studies have demonstrated that alkaloids have pharmacological effects and could be associated with inhibition of nucleic acid, protein, and membrane phospholipids biosynthesis (Shelton, 1991).

The mechanism of action of quaternary alkaloids such as berberine and harmane is linked to their ability to intercalate with DNA **(**Phillipson and O'Neill, 1987). Elsewhere is reported that alkaloid have common biological properties such as cytotoxicity, analgesic, antispasmodic, and antibacterial properties (Yadav and Agarwala, 2011). Nonetheless, Saxena *et al*., (2013) reported in their review that alkaloids have many pharmacological activities including antihypertensive, antiarrhythmic effects, antimalarial activity, anticancer action, stimulant property, analgesis, and antimicrobial (antifungal and antibacterial) activity. This probably explains the reason why the plants containing this group of natural products displayed antibacterial and antifungal activity.

Sterols may also be responsible for the observed antibacterial and antifungal activity demonstrated by these selected plant species extracts. Earlier studies have shown that sterols posses antibacterial, antifungal, antimycotic activity and act as inhibitors of tumor promotion *in vivo* (Yasukawa *et al*., 1991). Sterols were found to inhibit tumor promotion in two-stage carcinogenesis in mice and anti-inflammatory activity after topical application (Kasahara *et al*., 1994). Sterols exhibit inhibitory effect on HIV reverse transcriptase (Akihisa *et al*., 2001). Sheikh *et al.,* (2013) reported that cardiac glycosides are stimulant in body when body cardiac fail. Cardiac glycosides have strong activity on the heart and have been used in the treatment of heart failure and have pesticidal properties (Okwute, 1992).

Presence of flavonoids in the plant species may also be partly responsible for the observed antimicrobial activities (Table 4). Flavonoids being phenolic compounds are water soluble antioxidants and free radical scavengers which are capable of preventing oxidative cell damage and have strong anticancer activity (Okwu, 2004). Alan and Miller, (1996) reported the ability of flavonoids to scavenge hydroxyl radicals, superoxide anions and lipid peroxide radicals. Catechins (strong antioxidants), the most reduced form of the C_3 unit in flavonoid compounds occur in oolong green teas,that is why they have important dietary additive significance in food (Kaufman *et al.,* 1999). Sheikh (2013) reported that flavonoids are good against oxidants, allergies, inflammation, platelet aggregation, microbial ulcers, hepatoxins, viruses and tumors. Harborne and William (2000) reported that flavonoids possess useful properties such as anti-inflammatory, estrogenic enzyme

inhibition, antimicrobial, hypoglycemic, anti-allergic, antioxidant vascular, cytotoxic and anti-tumour activities. This forms the basis for use of these plants species by subsistence farmers in the management of human diseases.

Phenolic compounds are reported to be toxic to microorganism (Savoia, 2012; Adeogum *et al*., 2014). Phenolic compounds are most ubiquitous groups of plants metabolites which posses biological properties including anti-apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, natural antioxidant, cardiovascular protection, cell proliferation activities, improvement of endothelial function as well as inhibition of angiogenesis (Ali *et al.,* 2008; Yadav and Agarwala 2011). Sexana *et al.,* (2013) reported that phenol is a strong antioxidant. Phenol also increases bile secretion, anti-plasmodic, anti-depressant activities, anti-ulcer, purgative, stomachache, constipation, decrease blood pressure, cholesterol reduction; reduce lipid level and antimicrobial activities (Gibson *et al*; 1998; Mathai 2000; Sexana *et al.,* 2013).

Saponin compounds are reported to be good candidates for treating fungal and yeast infections, so they serve as natural antibiotics and fight against infections as well as microbial invasion in the body (Sheikh *et al.,* 2013). Somkuwar and Kamble (2013) reported that saponins are traditionally, used as detergents, pesticides, molluscicides and a foaming agent. Saponins are known to produce anti-inflammatory activity and erythrocyte haemolysis (Ngulde *et al.,* 2013). Triterpene saponins have shown to be effective in diseases conditions such as asthma, diabetes, atherosclerosis and cancer (Khan *et al*., 2011; Ngulde *et al.,* 2013). Sodipo *et al.,* (2000) reported that saponins produce inhibitory effects on inflammation, precipitation and coagulation of red blood cell, formation of foams in aqueous solution, hemolytic activity, and cholesterol binding and bitterness properties. Number of saponin are known to protect plants from insects attack as well as having antimicrobial, antifungal, and antiviral activities and have been used as anti-protozoan, hypocholesterolaemic and anti-carcinogenic agents (Takechi *et al.,* 1999; Saxena *et al.,* 2013). Thus, these phytochemicals might be responsible for the healing properties of the Plants as claimed by the subsistence farmers and traditional healers in Mbulu.

Terpenes are reported to be active against many bacterial infections (Barre *et al* 1997; Sheikh *et al* 2013). Somkuwar and Kamble (2013) reported that terpenoids are responsible for analgesics and inflammatory activities. Anti-feedants, anticarcinogenic, antimalarial, anti-ulcer and antimicrobial activities have also been reported (Dudareva *et al.,* 2004). The presence of these compounds in the plant species may contribute to the observed antibacterial, antifungal and anti-pest activity.

4.0 CONCLUSION

All crude extracts of the five plant species under investigation exhibited concentration-dependent activity against gram-positive, gram-negative bacteria and fungi (yeasts and moulds). Highly pronounced antimicrobial activity was displayed against fungi (yeast and mould), which serves as a clear indication of the potentials in these extracts as antifungal agents.

The study provides a pilot assessment of the plant species on their microbial potency. Although the plants are used locally, their active doses for different treatments are uncertain and hence they need scientific standardization. Since the activity may be a function of number of factors, the uncertainty on the source of bioactivity in the extracts need to be established. The findings from this study, therefore, form a baseline for further research for isolation, purification and characterization of pure bioactive compounds for further biological studies. More tests on the isolated pure compounds to establish the source of activity and hence structural modification of the compound for best activity is needed. Therefore, there is a need to promote collaboration between the traditional practitioners and the medical health care providers for experience and knowledge sharing for promotion of proper uses of these plants to control various diseases.

5.0 ACKNOWLEDGEMENTS

This work was sponsored by the Tanzania Commission for Science and Technology (COSTECH) through project No. CST/SC.327/952/3/2013 to which the authors are very indebted. The authors are also grateful to the Departments of Medicinal Chemistry and Microbiology, School of Pharmacy and Institute of Traditional Medicine (ITM), Muhimbili University of Health and Allied Science (MUHAS) for providing the laboratory facilities to carry out the work. Mr. E.S Marandu is acknowledged for the logistical and technical support.

Table 1: Cytotoxicity of ethanol crude extracts from five plants species

Plant Species	LC_{50} (µg/mL)	LC_{90} (µg/mL)	Regression Equation	R^2
PDL	4.57	88.25	y = 3.0963x + 2.9755	0.9522
PDR	34.74	726.09	y = 3.03x + 0.3312	0.8562
GSR	133.4	6166.22	y = 2.4026x - 0.1059	0.8898
CGR	71.09	1581.28	y = 2.9692x - 0.4985	0.9033
OFR	28.08	3904.41	y = 1.867x + 2.2957	0.9475
LNL	119.42	5286.40	y = 1.1140x + 4.1476	0.8459
PP	3.39	100.61	y = 2.7171x + 3.5586	0.9089

Legend: PDL = *P. dodecandra* (leaf); PDR = *P. dodecandra* (root); CGR = *C. geometrium* (root); GSR = G. *sylverstre* (root); OFR = *O. filamentosum* (root); LNL = *L. nepetifolia* (leaf) and PP = Potassium Permanganate.

Table 2: MIC and Mean Inhibitory Zone of Five Plant Species against Six bacterial Species

PLANT SPECIES	*E. coli*		*S. typhimurium*		*P. aeruginosa*		*K. pneumonia*		*S. Aureus*		*S. Dysentariae*	
	Mean IZ	MIC	Mean IZ	MIC	Mean IZ	MIC	Mean IZ	MIC	Mean IZ	MIC	Mean IZ	MIC
PDL	10±1	125	29.7±1.5	125	19±1	75	13.7±1.5	125	x	x	x	x
PDR	x	x	11.7±1.5	125	x	x	x	x	15±1	125	x	x
LN	15±2	125	x	x	10±1	125	10.7±1.5	125	15.3±1.5	75	10±1	125
CG	21±2	37.5	20±2.6	75	x	x	11.7±1.5	75	10±1	125	x	x
OF	15±1	125	x	x	x	x	11±2	125	19.7±1.5	125	11.3±1.5	125
GS	x	x	19±2	125	13±1	125	x	x	15.3±1.5	75	x	x
Gentamycin (Standard)	39.3±3.2	9.4	40.7±3.2	9.4	41±2.6	9.4	40.3±1.5	9.4	40.3±2.1	9.4	39.7±1.5	9.4

Legend: PDL = *P. dodecandra (*leaves*)*, PDR = *P. dodecandra (*roots), LN = *L. Nepetifolia*, CG = *C. geometrium*, OF = *O. filamentosum*, GS = *G. sylverstre*, IZ = Inhibition Zone in mm, MIC = Minimum Inhibition Concentration (mg/mL) and X = No activity

Table 3: MIC and Mean IZ of Selected Plant Species against Three Fungal Species

Plants Species	*A. niger*		*C. albicans*		*C. neofomans*	
	Mean IZ	MIC	Mean IZ	MIC	Mean IZ	MIC
PDL	x	X	9.3± 2.2	125	19.7± 2.1	9.4
PDR	15.0± 1.7	37.5	11.0±1.7	75	9.0± 1.6	125
LN	15.3±2.1	18.8	21.0±1.7	75	16.7±0.8	18.8
CG	9.3±0.8	125	10.0±1.4	75	12.7±0.8	37.5
OF	x	X	12.3±0.8	75	10.3±1.4	125
GS	x	X	9.3±2.2	125	10.0±1.4	125
Fluconazole Standard	41.0±1.4	9.4	39.7±2.1	9.4	39.3±0.8	9.4

Legend: PDL = *P. dodecandra (*leaves*)*, PDR = *P. dodecandra (*roots), LN = *L. Nepetifolia*, CG = *C. geometrium*, OF = *O. filamentosum*, GS = *G. sylverstre* = Inhibition Zone in mm, MIC = Minimum Inhibition Concentration (mg/mL) and X= No activity

Table 4: Qualitative Analysis of Phytochemical Constituents of Plant Species

	Phytochemical Constituents								
Plant species/part	**Saponins**	**Alkaloids**	**Sterols**	**Tritepenoids and terpenes**	**Phenol**	**Tannins**	**Flavonoids**	**Glycosides**	**Coumarins**
P.dodecandra (leaf)	+++	++	+++	+++	+	_	++	++	_
P.dodecandra (root)	+++	_	+++	+++	++	_	+++	+	-
L.nepetifolia (leaf)	+	+++	++	+	++	++	++	++	+++
O. filamentosum (root)	+	+++	+++	+	_	+++	+++	+	++
C. geomentrum (root)	++	_	_	_	_	++	+++	+	_
G. sylvestre (root)	++	++	+++	+++	++	_	-	+	_

Legend: (-): Absence of phytochemical compounds; the extract remains clears with no clears change. (+): The trace of phytochemical; faint changes against dark background, (++): Presence of phytochemical; definite colour change noticed, (+++): Intense presence of phytochemical; strong change noticed.

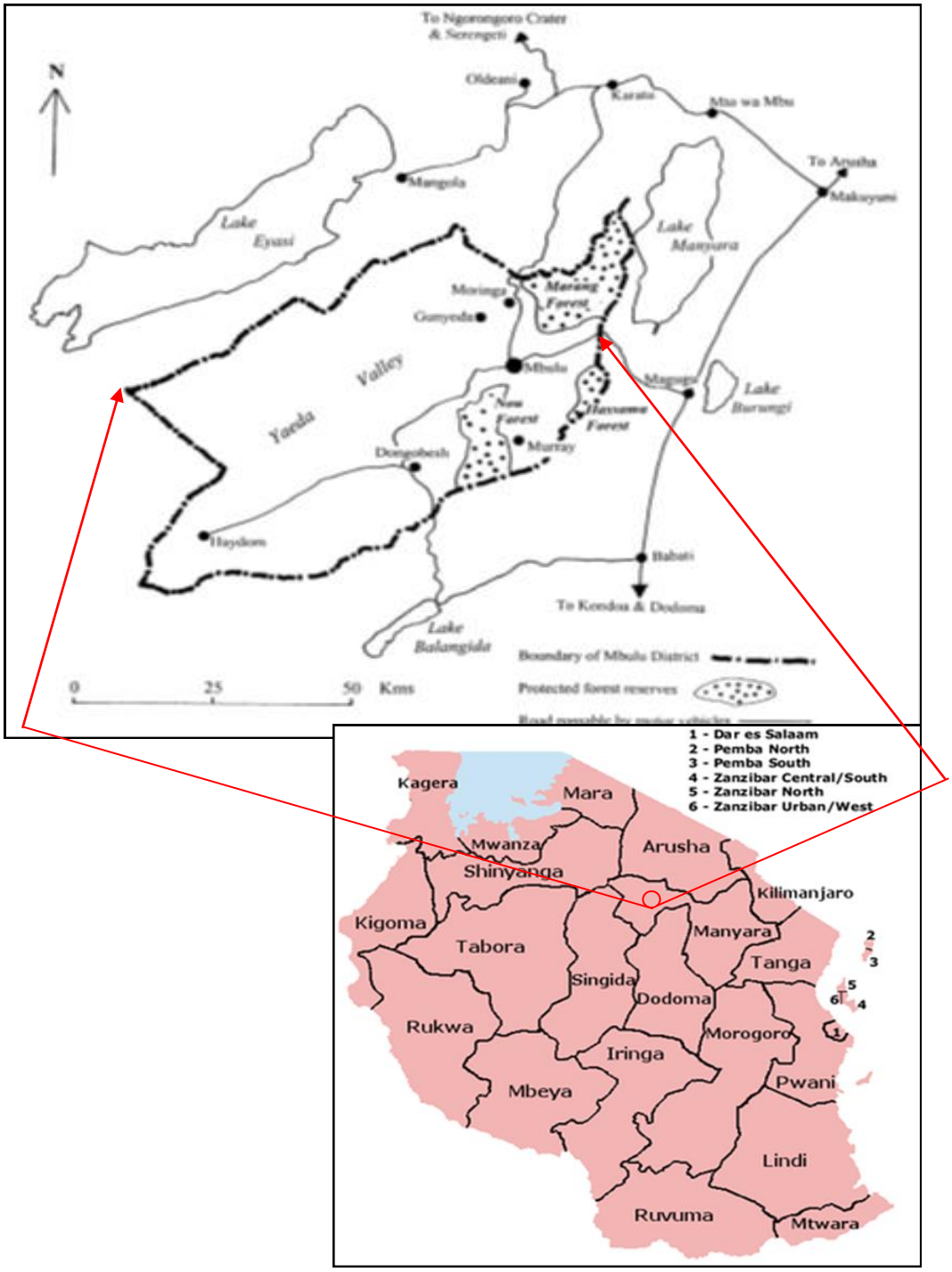

Fig. 1: Map of Tanzania showing Mbulu District (Modified from Google Earth)

6.0 REFERENCES

1. Adeogum OO, Adekunle AA and Ebabhi AM. Survey and phytochemical analyses of plants uses for the treatment of childhood diseases in Ojo Lga, Lagos State. International Journal of Herbal Medicine, 2014; 2 (2): 109-114.
2. Ahmad I, Aqil F and Owais M. Mordern phytomedicine, turning plants into drugs. Willey- VCH Verlag GMbH and Co KGaA, Weinheim. 2006
3. Ahmad I, Aqil F and Owais M. eds. Modern phytomedicine: Turning Medicinal plants into drugs. Wiley-VCH Verlag GmbH & Co. KGaA, 2006. pp 41, 59 and 263.
4. Ahmed IA, Kiyawa SA, Maryam TA, Kutama AS and Isah IB. Effects of

Pre-harvest application of Lamda-cyhalothrin and Neem seed extracts in the management of Cowpea bruchid, Callosobruchus maculates Fab.(Coleoptera:Bruchidae) on stored cowpea. Global Advanced Research Journal of Agricultural Science, 2014; 3 (3): 100-105.

5. Ajoy G and Padma G. Brine shrimp cytotoxic activity of 50% alcoholic extract of Croton bonplandianum baill. Asian J Pharm Clin Res, 2013; 6 (3): 40-41
6. Akihisa T, Ogihara J, Kato J, Yasukawa K, Ukiya M, Yamanouchi S and Oish K. Inhibitory effects of triterpenoids and sterols on human immunodeficiency virus-1 reverse transcriptase. Lipids, 2001; 36: 507-512.
7. Alan L and Miller ND. Antioxidant flavonoids: Structure, function and clinical Usage. Alt Med. Rev. 1996; 1:103-111.
8. Ali SS, Kasoju N, Luthra A, Singh A, Sharanabasava H, Sahual A and Bora U. Indian medicinal herbs as source of antioxidants. Food Res. Int. 2008; 41: 1-15.
9. Aziz A, Shawn MMAK, Rahman S, Islam T, Mita M, Faruque A and Rana S. Secondary metabolites, antimicrobial, brine shrimp lethality and 4th instar Culex quinquefasciatus mosquito larvicidal screening of organic & inorganic root extracts of Microcos paniculata. IOSR Journal of Pharmacy and Biological Sciences. 2013; 8 (5): 58-65.
10. Baraza LD, Moshi CC and Nkunya MHH. Chemical constituents' biological activity of tree Tanzanian wild mushrooms species. Taz. J. Sci. 2007; 33: 1-8.
11. Barre JT, Bowden FB, Coll JS, Jesus J, Fuente VE and Janairo G.S. Bioactive triterpenes from Lantana camara. Phytochemisrty. 1997; 45: 321-324.
12. Barros L, Calhelha RC, Vaz JA, Ferreira ICFR, Baptista P and Estevinho LM. Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms. Eur. Food Res. Technol. . 2007; 225: 151-156.
13. Björnsen L. A history under Siege: Intense agriculture in the Mbulu highlands, Tanzania, 19th century to the present. The journal of the international African institute. 2006;.76 (4). 60-71.
14. Brownlee HE, McEuen R, Hedger J and Scott IM. Anti-fungal effects of cocoa tannin on the witches' broom pathogen Crinipellis perniciosa. Physiol. Mol. Plant Patho. 1990; 36: 39-48.
15. Buvaneswari K, Ramamoorthy D and Velanganni J. Preliminary phytochemical and antimicrobial Activity studies on the leaves of the Indian plant Thevetia neriitfolia Juss. World Journal of Agricultural Sciences. 2011; 7(6): 659-666.
16. Chakraborty D and Chakraborti S, Bioassay-guided isolation and identification of antibacterial and antifungal component from methanolic extract of green tea leaves (Camellia sinensis). Research Journal of Medicinal Plant. 2010;.4(2): 78-86.
17. Darokar M, Mathur A and Dwired S. Detection of antibacterial activity in the floral petals of some higher plants. Curr. Sci. 1998;75 (3):187-189
18. Dudareva N, Pichersky E and Gershenzon J. Biochemistry of plant volatiles. Plant Physiology. 2004; 135: 1893–1902.
19. Ebana RUB, Madunagu BE and Etok CA, Anti-microbial effect of Strophantus hipides Secamone afzeli on some pathogenic bacteria and their drug Research strain. Nigeria Journal of Botany. 1993; 6: 27-31.
20. Fabio G, Zarrell M, Giordano M and Zarrell A. C-4 Gem-Dimethylated oleanes of Gymnema sylvestre and their pharmacological activities. Molecules. 2013; 18: 14892-14919.
21. Gibson EL, Wardel J and Watts CJ. Fruit and vegetable consumption, nutritional knowledge and beliefs in mothers and children. Appetite. 1998; 31: 205-228.
    Harborne JB and Williams CA. Advances in flavonoid research Since 1992. Pytochemistry. 2000; 55: 481-504.
22. Haslam E. Natural polyphenols (vegetable tannins) as drugs: possible modes of action. J. Nat. Prod. 1996; 59: 205-215.
23. Hasler CM and Blumberg JB. Symposium on phytochemicals: biochemistry and physiology. Journal of Nutrition. 1999; 129: 7565-7575
24. Heinrich M, Barnes J, Gibbons S and Williamson EM. A text book of

fundamentals of pharmacognosy and phytotherapy. Elsevier, USA. 2004, 1st Edition,
25. Iqbal Z, Perzer MA, Ahmad S, Iftikhar Y, Yasin M, Nawaz A, Ghazanfar MU, Dasti AA and Saleen A. Determination of minimum inhibitory concentration of fungicides against Fasarium mangiferae. Pak. J. BOT. 2010; 41(5): 3525-3532.
26. Jaiganesh KP and Arunachalam G. Preliminary phytochemical screening and antimicrobial potential and Pterosperum canescens Roxb., (Sterculiaceae) Bark. International Journal of Biological and Pharmaceutical Research. 2013; 4 (3): 212-215.
27. Jamil M. 2010. Screening of selected plant species of Pakistan for their pharmacological activities. PhD Thesis, Quaid-i-Azam University, Islamabad. pp 166.
28. Jeyachandran R and Mahesh A. Antimicrobial evaluation of Kigelia Africana (Lam). Res. J. Microbial. 2007; 2 (8): 645-649.
29. Joshi S and Sati SC 2012. Antifungal Potential of Gymnosperms: A Review, in Sati SC, Belwal M, ed. Microbes: Diversity and Biotechnology. pp. 333-34
30. Kaufman BP, Cseke JL, Waber S, Duke AJ and Brielmann LH. 1999. Natural products from Plants, , CRC press LLC, USA, 1st Edition
31. Khan MA, Chen HC, Tania M and Zhang DZ. Anticancer activities of Nigella sativa (Black cumin). Afr. J. Tradit. Complement Altern. Med. 2011; 8 (5): 226-232.
32. Khanna VG and Kannabiran K. Larvicidal effect of Hemidesmus indicus, Gymnema sylvestre, and Eclipta prostrata against Culex qinquifaciatus mosquito larvae. African Journal of Biotechnology. 2007; 6 (3): 307-311
33. Kidukuli AW, Mbwambo ZH, Malebo HM, Mgina C and Mihale MJ. In vivo anti viral activity, protease inhibition and brine shrimp lethality of selected Tanzanian wild edible mushrooms. Journal of Applied Biosciences. 2010; 31: 1887-1894.
34. Kokate CK. Practical pharmacognosy Vallabh Prakashan, Delhi, 1996. 4th edition
35. Kumar VR and Pradeep P. Primary and secondary analysis of some medicinally pontent Plants. International Research Journal of Pharmacy. 2011; 2 (6): 166-168.
36. Lui RH. Health benefits of fruit and vegetables are from additive and Synergistic Combinations of Phytochemicals. American Journal of clinical Nutrition. 2003; 78:5175-5205
37. Magoggo JP and Meliyo JL. 1994. Land resources inventory and land suitability assessment of Mbulu , district region, Tanzania NSSR report R5, National soil service. 75.
38. Mahmoud MAS. 2004. Bioactive secondary metabolites from marine and terrestrial bacteria: Isoquinolinequinones, Bacterial Compounds with a Novel Pharmacophor. PhD dissertation. Georg-August-Universität zu Göttingen, Germany
39. Mann A, Banso A and Clifford LC. An antifungal property of crude plant extracts from Anogeissus leiocarpus and Terminalia avicennioides. Tanzania Journal of Health Research. 2008; 10 (1) :34-38
40. Maobe MAG, Gitu L, Gatebe E, Rotich H, Karanja PN, Votha MD, Nderitu WT and Kungu W. Antifungal activity of eight selected herbs used for the treatment of diabetes, Malaria and Pneumonia in Kisii region, Southwest Kenya. World Journal of Medical Science. 2013; 8(1): 74-78.
41. Mathai K. 2000. Nutrition in the adult years in Krause's food, nutrition, and diet therapy, 10th edition, 271: 274-275.
42. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE and Mclaughlin JL. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982; 45, 31-34.
43. Mungenge C, Zimudzi C, Zimba M and Nhiwatiwa T. Phytochemical screening, cytotoxicity and insecticidal activity of the fish poison plant Synaptolepis alternifolia Oliv. (Thymelaeaceae). Journal of Pharmacognosy and Phytochemistry. 2014; 2 (5): 15-19
44. Nathathe B and Ndip R. Bioactivity of Hydana Africana on selected bacterial pathogens: Preliminary phytochemical screening. African Journal of Microbial Research. 2011; 5 (18): 2820-2826.
45. National Committee for clinical Laboratory Standards. Method for

dilution antimicrobial susceptibility test for bacteria that grow aerobically. National Committee for clinical Laboratory Standards. Wayne. 1997. 4rd edition.
46. Ngoci NS, Ramadhan M, Ngani MS, Leonard OP. Screening for antimicrobial activitiy of Cissampelos papeira methanol root extract. European journal of medicinal plants. 2014; 4 (1): 45-51.
47. Ngulde SI, Sandabe SI, Sandabe UK, Tijjani MB, Barkindo AA and Hussaini IS.. Phytochemical constituents, antimicrobial screening and acute toxicity studies of the ethanol extract of Carissa edulis Vahl. root bark in rats and mice .American Journal of Research Communication. 2013; 1 (9): 99-110.
48. Ogbonna JDN, Ezema EB, Obimma CP and Agibo MO. (2013). Phytochemical properties of methanol extract and antimicrobial study of less polar fractions of Lotanthus micranthus (Linn) parasitic on Alstonia boonei. The Pharma Innovation Journal 2(8) :83-88.
49. Okigbo N, Anuagasi C, Amadi J. Advances in selected medicinal and aromatic indigenous to Africa. Journal Medicinal Plants Research. 2009; 3 (2): 86-95.
50. Okwu DE. Phytochemicals and vitamin C content of indigenous spices of Southeastern Nigeria. J. Sustain Agric. Environ. 2004; 6: 30-37.
51. Okwute SK. Plants derived pesticidal and antimicrobial agents for use in agriculture: A review of phytochemical and biological studies on some Nigerian plants. J. Agri. Sc. Tech. 1992; 2 (1): 62-70.
52. Omoregbe RE, Ikuebe OM and Ihimire IG. Antimicrobial activity of some medicinal plants extracts on Escherichia coli, Salmonella paratyphi and Shigella dysenteriae. African Journal of Medicine and Medical Sciences, 1996; 25 (4):373-375.
53. Omwenga EO and Paul OO. Antimicrobial evaluation of the methanol bark extracts of Plumbago dawei Rolfe, a local species used by the Samburu community, Wamba, Samburu district, Kenya for the treatment of diarrheal ailments. Malaysian Journal of Microbiology. 2012; 8(4): 248-252.
54. Peteros NP and Mylene MM. Antioxidant and cytotoxic activities and phytochemical screening of four Philippine medicinal plants. Journal of Medicinal Plants Research. 2010; (5): 407-414.
55. Phillipson JD and O'Neill MJ. New leads to the treatment of protozoal infections based on natural product molecules. Acta Pharm. Nord. 1987; 1:131-144.
56. Qwarse M. 2015. Assessment of bioactivity of selected plants against pests and microbes from Agro-pastoral communities in Mbulu district, Tanzania, MSc. Thesis, Open University of Tanzania, Dar es Salaam
57. Rahman SM, Alam M, Amin MR, Fakruddin MD, Shahid-Ud-Daula AFM and Siddiqui R. Antimicrobial activity and brine shrimp toxicity of methanolic whole plant extract of Boerhavia repens L. (Family: Nyctaginaceae). International Journal of Phytopharmacy. 2014; 4(6): 135-139.
58. Rajeh MAB, Kwan YP, Zakaria Z, Latha LY, Jothy SL and Sasidharan S. Acute toxicity impacts of Euphorbia hirta L extract on behavior, organs body weight index and histopathology of organs of the mice and Artemia salina. Pharmacognosy Research. 2012; 4(3): 170-177.
59. Salvonia D. Plant-derived antimicrobial compounds: Alternatives to antibiotics future. Microbiology. 2012; 7(8): 979-990.
60. Sam TW. Toxicity testing using the brine shrimp: Artemia salina in Colgate SM, Molyneux RJ. (eds.). Bioactive natural products: Detection, isolation and structural determination. CRC Press, USA. 1993.
61. Sarker AD, Lafif Z and Gray AI. 2006. Natural product isolation. Humana Press Inc. Second edition, Totowa, New Jersey.
62. Sati SC, Joshi S and Kumar P. Antibacterial activity of Kumaum Himalaya Biota orientals L. Leaf. African Journal of Microbiology Research. 2014; 8(6): 603-608.
63. Sati SC and Joshi S. Aspects of antifungal potential of ethnobotanically known medicinal plants. Res. J. Med. Plant. 2011; 5(4):377-391.
64. Saxena M, Saxena J, Nema R, Singh D and Gupta A. Phytochemistry of medicinal plants. Journal of

Pharmacognosy and Phytochemistry. 2013; 1 (6): 168-182.
65. Screeshman LS and Nair BR. Brine shrimp lethality assay in two species of Biophrtum (Oxalidaceae). Internal Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(4): 582-586.
66. Sheikh N, Kumar Y, Misra AK and Pfoze L. Phytochemical Screening to validate the ethnobotanical importance of Root tubers of Dioscorea species of Meghalaya, North East India. Journal of medicinal Plants Studies. 2013;.1(6): 62-69.
67. Shelton RM. Aloe vera: Its chemical and therapeutic properties. Int. J. Dermat. 1991; 30: 679-683.
68. Sodipo OA, Akinyi JA and Ogunbamosu JU. Studies on certain characteristics of extracts of bark of Pansinystallia macruceras (kschemp) picrre exbeille. Global J. Pure Appl. Sci. 2000; 6:83-87.
69. Somkuwar DO and Kamble AV. Phytochemical Screening of ethanolic extracts of stem , leaves, flower and seed kernel of Mangifera indica L. Int. J. Pharm. Bio. 2013; 4(2): 383-389.
70. Takechi M, Matsunami S, Nishizawa J, Uno C and Tanaka Y. Haemolytic and antifungal activities of saponins or anti-ATPase and antiviral activities of cardiac glycosides. Planta Medica. 1999; 65: 585– 586.
71. Vipra A, Desai SN, Junjappa RP, Roy P, Poonacha N, Ravinder P, Seriran P and Radmanabha S. Determining the minimum inhibitory concentration on bacteriophages: Potential advantages. Microbiology Team, Gangagen Biotechnologies PVT Ltd. 2013; 3: 180-190.
72. Wahi AL, Singh A and Singh AK. Determination of minimum inhibitory concentration (MIC) of some novel triazole derivative. International Journal of Research in Pharmacy and Chemistry. 2011;1(4):1108-1114.
73. Yadav RNS and Agarwala M. Phytochemical analysis of some medicinal plants. Journal of Phytology. 2011;3(12):10-14.
74. Yasukawa K, Takido M, Matsumoto T, Takeuchi M and Nakagawa S. Sterol and Triterpene Derivatives from Plants Inhibit the Effects of a Tumor Promoter, and Sitosterol and Betulinic Acid Inhibit Tumor Formation in Mouse Skin Two-Stage Carcinogenesis. Oncology. 1991;48: 72-76
75. Zhao GX, Hui YH, Hui YH, Rupprecht JK, McLaughlin JL nd Wood KV. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of Asimina triloba, Journal of Natural Products. 1992;55:347-356.
76. Zimudzi C. African potato (Hypoxis Spp): Diversity and comparison of the phytochemical profiles and cytotoxicity evaluation of four Zimbabwean Species. Journal of Applied Pharmaceutical Science. 2014;4(4): 079-083.