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Original Research

Ethnobotanical survey and toxicity evaluation of medicinal plants used for fungal remedy in the Southern Highlands of Tanzania

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ABSTRACT

Background/Aim: Some of the antifungal drugs used in the current treatments regime are responding to antimicrobial resistance. In rural areas of Southern Tanzania, indigenous people use antifungal drugs alone or together with medicinal plants to curb the effects of antibiotic resistance. This study documented ethnobotanical information of medicinal plants used for managing fungal infections in the Southern Highlands of Tanzania and further assess their safety. **Materials and Methods:** Ethnobotanical survey was conducted in Makete and Mufindi districts between July 2014 and December 2015 using semi-structured questionnaires followed by two focus group discussions to verify respondents' information. Cytotoxicity study was conducted on extracts of collected plants using brine shrimp lethality test and analyzed by MS Excel 2013 program. **Results:** During this survey about 46 plant species belonging to 28 families of angiosperms were reported to be traditionally useful in managing fungal and other health conditions. Among these, *Terminalia sericea*, *Aloe nutii*, *Aloe lateritia*, *Zanthoxylum chalybeum*, *Zanthoxylum deremense,* and *Kigelia africana* were frequently mentioned to be used for managing fungal infections. The preparation of these herbals was mostly by boiling plant parts especially the leaves and roots. Cytotoxicity study revealed that most of the plants tested were nontoxic with $LC_{50} > 100$ which implies that most compounds from these plants are safe for therapeutic use. The dichloromethane extract of *Croton macrostachyus* recorded the highest with LC_{50} value 12.94 μg/ml. The ethnobotanical survey correlated well with documented literature from elsewhere about the bioactivity of most plants. **Conclusions:** The ethnobotanical survey has revealed that traditional healers are rich of knowledge to build on for therapeutic studies. Most of the plants are safe for use; and thus can be considered for further studies on drug discovery.

KEY WORDS: Ethnobotanical, fungal, brine shrimp test, medicinal plants, traditional medicine

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INTRODUCTION

The history of mankind has continuously remained interlocked to the surrounding environment. The first civilizations realized that there were plants with healing potential. The value of plants has a long history in saving human beings cutting across different cultures in the world [1]. Utilization of medicinal plants by individuals lies on the knowledge accumulated through the interaction of people with the environment and the diffusion of information, traditionally transmitted orally through subsequent generations [2]. In the contemporary world of conventional medicine, the practice of herbal medicine has attracted more attention and is becoming accepted globally [3]. Traditional medicine is not well documented in most African societies [4]. However, the practices and resources have been orally transferred from one generation to another thus limiting its reliability.

Documenting the indigenous knowledge through ethnobotanical studies is important for sustainable utilization of medicinal plants in drug discovery. Several active compounds have been discovered from plants based on ethnobotanical information, some used directly as therapeutic drugs [3]. Therefore, the focus of the study was to collect and document information on the use of antifungal medicinal plants and their therapeutic practices among the Hehe and Kinga tribe in Southern Highland of Tanzania. The information could further help scientific research in drug development.

MATERIALS AND METHODS

Study Area

The study was conducted in Mufindi District found in Iringa Region and Makete District based in Njombe Region. Makete District is one of the six districts of Iringa Region and is located in the Southern Highlands of Tanzania about 115 km from the regional headquarters (Figure 1). It is situated within 9°15'0" S 34°10'0" E [5]. Mufindi district on the other hand lies between 08°35′40″S 035°17′20″E. Both districts are dominated by Hehe, Kinga and Bena ethnic tribes. Furthermore, these districts experience high levels of migration and mobility (61.4%) caused by seasonal workers to numerous plantations in the areas and being a logistical hub for transport infrastructural facilities by road and railway (Tanzania-Zambia route) [6]. These unique dynamics increase the risk for HIV transmission in the communities. Most of the livelihoods are from agriculture which is the major source of subsistence, occupying about 80 % of the households in the districts [5]. Other activities include livestock keeping, timber production, and petty businesses at small scale. Most household members are thus compelled to engage in multiple jobs and activities to make a living [5].

During the ethnobotanical survey that was done between July 2014 and September 2015 semi-structured questionnaire was used as data collection tools to interview traditional health practitioners, elders and selected villagers who have knowledge on medicinal plants. This study employed a purposive sampling, in which selection of respondents do only focus to people who are considered by the community as having exceptional knowledge about the use of plants such as traditional healers, herbalists and elders. The questionnaire aimed to collect and document ethnobotanical information of plants that are used to treat various infections including fungal infections. Documentation of plants, parts used and their preparations whenever possible was done. Focus group discussion was employed to validate information collected using questionnaire method.

Collection of Plant Materials

Identification of plant species was done by the botanist from the Department of Botany, University of Dar es Salaam, Tanzania, and all voucher specimens were deposited at the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences. Collection of the identified plants was aided by the traditional health practitioners and elders. Decision on which plant and/or part of plant to be collected for further studies was mainly influenced by the information given by respondents in the field validated first by focus group discussion and by literature.

Reagents

Absolute ethanol, dichloromethane, and petroleum ether were purchased from Fluka Chemie GmbH (Sigma-Aldrich®, Zwijndrecht, Netherlands), dimethyl sulfoxide (DMSO) was purchased from Sigma® (Poole, Dorset, UK) while sea salt was prepared locally by evaporating water collected from the Indian Ocean, along the Dar es Salaam Coast.

Extraction and Concentration

Plant materials from the field were cut into small pieces, air-dried and ground using a machine grinder consequently soaked, sequentially using petroleum ether, dichloromethane, and ethanol for 48 h for each solvent. The method of percolation was employed during extraction process. The crude extracts were obtained by concentrating the filtrate in vacuo using a rotary evaporator with the bath temperature maintained at 40°C. The crude extract obtained was placed in the refrigerator for few hours and then subjected to freeze drier to remove solvent that could have remained.

Brine Shrimp Lethality Test

The brine shrimp lethality assay was used as an indication for bioactivity of different tested plant extracts as well as investigation for toxicity [7,8]. Artificial seawater was prepared by dissolving 3.8 g of sea salt in 1 L of distilled water. Brine shrimp eggs (2 g) were added and left for 24 h to hatch in light condition. Stock solutions (40 mg/mL) of all extracts were dissolved in DMSO. Different levels of concentrations (240, 120, 80, 40, 24, 8, 4.5, 3, 1.5 and 1 μg/ml) were prepared by drawing different volumes from the stock solutions and then added into vials, each containing ten brine shrimps larvae. The volume was adjusted with the prepared artificial seawater. Each level of concentration was tested in duplicate. The negative control contained brine shrimp, artificial seawater and DMSO (0.6%) only. The vials were incubated under light for 24 h. The dead larvae were counted and mean percentage mortality calculated.

Data Analysis

The mean percentage mortality was plotted against the logarithm of concentrations and the concentration killing 50% of the larvae (LC_{50}) were determined from the graph using Microsoft Excel 2013 computer software. Regression equation obtained enabled calculation of lethal concentrations, i.e., LC_{50}, LC_{16}, and LC_{84}. The 95% confidence interval was then calculated using method reported by Litchfield and Wilcoxon [9]. The results were used to document safety and cytotoxicity activity of plant extracts.

RESULTS

Ethnobotanical Survey

During the ethnobotanical survey, a total of 40 respondents (traditional healers, herbalists, and elders) were interviewed from the selected regions. 5 different villages in Njombe and Iringa regions were visited for the survey including three villages; Tambalang'ombe, Mayale, Kingege, and Ifwagi from Mufindi, Iringa region as well as Lupalilo and Maliwa villages of Makete district in Njombe region. These villages were chosen based on the information of registered or known traditional health practitioners obtained from the District Medical offices.

A total of 46 plant species used by the Hehe, Bena and Kinga tribe for the treatment of various microbial related ailments were documented [Table 1]. The plants represent about 28 families with the most prominent families being Euphorbiaceae (6 species), Combretaceae, and Rubiaceae (4 species each) and followed by Rutaceae, and Fabaceae (each with 3 species). Most of the ethnobotanical information were related to fungal infections since the study focused on documenting plants that were used in managing fungal infections among these ethnic groups. Out of 46 reported plant species, 14 (32%) had similar cited antifungal activity while 8 (18%) of plant species traditionally used for managing other nonfungal infections in Mufindi and Makete districts were reported by the literature to have antifungal activity [Table 1].

Brine Shrimp Lethality Assay

The brine shrimp test is used as a preliminary test for testing toxicity of a plant and anticancer activity after a single dose administration. In this study, the LC_{50} values were clustered per Moshi *et al*., [95]. The LC_{50} of <1.0 μg/ml is considered highly toxic; LC_{50} 1.0-10.0 μg/ml is toxic; LC_{50} 10.0-30.0 μg/ml - moderately toxic; LC_{50} > 30 < 100 μg/ml - mildly toxic and LC_{50} > 100 μg/ml as nontoxic. Studies done by Moshi *et al*., [96,97] provided the evidence that plant extract with the LC_{50} <20 μg/ml could be a source for anticancer compounds. The results from this study revealed that most (77.1%) of the plants tested were nontoxic with LC_{50} value <100 [Table 2]. The present findings imply that most compounds from these plants were safe for therapeutic use. Among the tested plant extracts dichloromethane extract of *Croton macrostachyus* had moderate toxicity with LC_{50} value 12.94 μg/ml.

DISCUSSION

Ethnobotanical Survey

Plant-based traditional medicine system continues to play an essential role in primary health care for the wider communities irrespective of the locality. This work has revealed the potential herbal medicines used in managing fungal infection in Njombe and Iringa Regions which are leading in spread of HIV infection in Tanzania with about 14.8% and 9.1% HIV prevalence, respectively [6,94]. Association of opportunistic fungal infections and HIV have been reported from the early days of the HIV/AIDS pandemic in Tanzania and worldwide [98]. The majority of the people living with HIV/AIDS are susceptible to fungal and bacterial opportunistic infections due to immunity suppression [37]. Availability of fungal herbal medicines may subsidize the effect of antifungal drugs resistance and availability to patients due to recurring fungal infections. The findings showed that remedies used in these communities consisted of one or a combination of two or more plant species. According to the traditional health practitioners, combinations of different plant species increases the efficiency of medicine and improves the cure's power which could be due synergistic effects in treatment of various diseases. Most of plant species collected have been documented to be used in different African communities for the treatment of skin diseases [12]. Furthermore, the study noted that there was a wide use of the leaf part which could be considered as a good sign for the conservation of the environment and ensures sustainable utilization of plants.

Among the frequently mentioned plants, included *Terminalia sericea*, *Aloe nutii*, *Aloe lateritia*, *Zanthoxylum chalybeum*, *Zanthoxylum deremense*, and *Kigelia africana*. The claims on these plants have a special merit as they are also recorded in the literature to be useful in managing various microbial infections. Pharmacological studies by several authors have demonstrated the potency of the mentioned plants in terms of antifungal activity [12,16,21,27,30,81,99,100]. However, the proportion of claims made by traditional health practitioners in Makete and Mufindi districts concerning some of the plants documented in this study and which are supported by literature evidence of proven biological activity or similar ethnobotanical uses elsewhere is remarkable. The results also confirmed the supportive role of traditional health practitioners in offering health-care services to local communities in addition to available conventional medical cares.

Brine Shrimp Lethality Assay

Apart from efficacy, safety of herbal medicines is of paramount importance as little is documented about many plants that are used in traditional medicine. Findings from various studies have recommended brine shrimp assay as one of the methods for preliminary investigations of toxicity. This assay is also used in screening bioactive compounds from medicinal plants popularly used for several purposes and for monitoring the isolation of such biologically active compounds [101-103]. This work present few results from plant extracts that were tested for toxicity against brine shrimps. However, not all collected plant samples were screened for toxicity since during extraction yield was very little or none for some samples to be used for the testing. Findings obtained in this study showed that 77.1% of plant tested to be nontoxic supporting the popular use of medicinal plants by communities since they are regarded as safe therapeutic agents. Unlike other plants, *C. macrostachyus* exhibited high toxicity level that suggests its potential for anticancer agents. The LC_{50} of *C. macrostachyus* (12.94 μg/ml) is not statistically different to the standard anticancer drug

Table 1: List of medicinal plants reported for managing various diseases in Iringa and Njombe regions

Family	Botanical/common name	Part of the plant	Ethnobotanical preparation and use	Reported ethnopharmacology activity	Reported phytochemical profile or compound	Frequency of mention during FGD	Supporting literature
Acanthaceae	*Dicliptera laxata* (Hehe)	Leaves and roots	Roots are chewed as a stomach pain and coughs remedy Leaf decoction is drunk to treat fever, headache, rashes and itching	Antimicrobial Inflammatory Antinociceptive	No report	05	[10]
Aloaceae	*Aloe lateritia* Litembwembwe (Hehe) Lyusi (Kinga)	Leaves	Combined with other plant roots and use for washing the wounds for 7 days Leaves can be boiled and drunk or applied topically for fungal infections Leaves can also be used against typhoid and wounds	Antimicrobial	Alkaloids, phenolic compounds, tannins, terpenoids	25	[11,12]
	Aloe nutii Litembwetembwe (Hehe)	Leaves and roots	Grind the leaves and soak, for roots grind into powder and take a tea spoon. It can also be mixed with *Toddalia asiatica* and mngalanga to stop diarrhea for HIV/AIDS patients The juice from leaves rubbed on the skin to treat ringworm Leaves decoction for diarrhea	No report	Alkaloids, phenolic compounds, tannins, terpenoids	17	[11]
Anacardiaceae	*Sorindeia madagascariensis* Muzingilizi (Bena)	Leaves, stem barks and roots	Grind the stem barks and smell for headache Root used for treatment of tuberculosis	No report	No report	10	No report
Apocynaceae	*Rauvolfia caffra* Mveriveri (Hehe)	Roots and stem barks	Roots decoction used for management of mental case and epilepsy Stem barks decoction used for rheumatism and chest pains	Antioxidant Antimicrobial	Alkaloid resperine, serpentine	09	[13,14]
Asteraceae	*Bidens pilosa* Lipuli (Hehe)	Leaves, roots and seeds	Leaves grounded and soaked to be gargled in the mouth-oral infection Decoctions of leaf powder for kidney problems, headache and blood clotting Leaves prepared as poultice for wounds and cuts	Anti-inflammatory, antifungal, antibacterial, antimalarial, antitumor Antihyperglycemic, antihypertensive, antiulcerogenic, hepatoprotective, antipyretic Immunosuppressive, antileukemic, antioxidant	Tannins, flavonoids, phlobatannins, terpenoids and cardiac glycosides	10	[10,11,15]

(*Contd...*)

Table 1: (*Continued*)

Family	Botanical/common name	Part of the plant	Ethnobotanical preparation and use	Reported ethnopharmacology activity	Reported phytochemical profile or compound	Frequency of mention during FGD	Supporting literature
Bignoniaceae	*Kigelia africana* Mfumbi (Hehe) Sausage tree (English)	Fruits, leaves and stem, root barks	Take the fruit sap apply over the wounded part for acute wounds Leaves and stem barks decoction used for treatment of STDs Fruits and barks decoction and powder for skin, fungal infections	Antibacterial Antioxidant Antiulcer Antifungal Antipyretic	Iridoids, flavonoids, naphthoquinones, meroterpenoid coumarin derivatives, lignans, sterols, furanones, furonaphthoquinones	16	[15-17]
Caesalpinaceae	*Ximenia caffra* Mtundwa (Bena) Mpingipingi (Hehe)	Roots and leaves	Roots decoction for treatment of hypertension, chest pain, infertility bilharzia and epilepsy A decoction of leaves is used as a remedy for malaria, coughs, toothache Pounded leaves are used as poultices for wounds and boils	Anti-infammatory Antigonococcal Antibacterial	Gallic acid, catechin, quercetin, kaempferol, terpenoids	07	[18,19]
	Hymenaea verrucosa Gaerth	Stem bark		No report	Terpenes	05	[20]
Celastraceae	*Elaeodendron buchananii* Muhulamwiko (Hehe)	Stem bark	Stem barks powder used for topical application against fungus	Antifeedant	Buchaninoside, glycoside, dihydroagarofuranoid sesquiterpene (mutangin)	08	[21,22]
Chrysobalanaceae	*Parinari curatellifolia* Msaula/msawola (Hehe)	Roots, stem barks and leaves	Leaves decoction for treatment of anemia Barks powder are used for vaginal douches, treatment of itching scalp	Antioxidant Antibacterial Antidiabetic	Phenols, flavoniods, sterols, terpenoids, carbohydrates and saponnins	12	[23,24]
Clusiaceae	*Garcinia buchananii* Mduma/mfilafila (Hehe)	Roots stem barks	Stem barks powder used against abdominal discomfort, pains An infusion from roots used as aphrodisiac and lotion for sores	Antidiarrheal Anti-inflammatory Antipropulsive motility Antiperistaltic	Biflavanones, flavonoids, steroids, alkaloids, tannins and phenols	15	[25,26]
	Garcinia acutifolia Baker Mfilafila/ Mduma (Hehe)	Leaves, stem barks and roots	An infusion from the roots is used as an aphrodisiac and as a lotion for lotion Stem barks decoction for venereal diseases and powder massaged for abdominal discomfort	No report	Xanthones, friedelin, stigmasterol	01	No report

(*Contd*...)

Table 1: (*Continued*)

Family	Botanical/common name	Part of the plant	Ethnobotanical preparation and use	Reported ethnopharmacology activity	Reported phytochemical profile or compound	Frequency of mention during FGD	Supporting literature
Combretaceae	*Terminalia sericea* Mpululu (Hehe)	Leaves, stem barks and root barks	Roots decoction for washing and apply on wounds and drinking-fungal infection Dried leaves and powdered to make decoction for dysentery Roots and leaves decoction for CD_4 boosting, syphilis, gonorrhea	Antimicrobial Anti-inflammatory Antioxidant	Anolignan B, Saponins, Glycoside, triterpene sericoside, β-sitosterol, β-sitosterol-3-acetate, lupeol, and stigma-4-ene-3-one	15	[27-31]
	Combretum zeyheri Mnavasenga (Hehe)	Roots and leaves	Roots and leaves used against ameba infections and abdominal Boil the roots and drink for Oesophageal candidiasis	Antifungal Antibacterial Antioxidant	Flavonoids	10	[30,32,33]
	Terminalia mollis Mupululu (Hehe)	Leaves, barks Roots	Roots, leaves or roots decoction for bilharzia, coughs, measles, rectal prolapse, and stomachache, HIV	Antioxidant Antimicrobial Antiplasmodial Anti-HIV	Tannins, triterpenes, flavonoids, gallic acid and saponins	14	[34-38]
Cucurbitaceae	*Cumumis dipsaceus* Mtango mwitu (Swahili)	Leaves and roots	Leaves and roots pounded and used as poultice for wound treatment	Antioxidant	Phenolics, flavonoids, tannins	09	[39,40]
Euphorbiaceae	*Psorospermum febrifugum* Mfwifwi (Hehe)	Leaves and roots	Leaves dried then grounded and smear on affected part-fungus Roots grounded and soaked in water for oral infection-as a mouth wash or gargle for tonsillitis	Antifungal Antitumor Cytotoxic Anticonvulsant	Steroids, acetylvismione F, prenylated bianthrone and 1, 8-dihydroxyanthraquinone	12	[41-44]
	Clutia abyssinica Mvuruku (Pare)	Leaves and roots	Roots prepared as hot infusions for kidney cleansing and roundworms	Antimicrobial	No report	05	[45]
	Eurphorbia candelabrum/trucalli Mlangali (Hehe)	Roots and latex	Latex is used against sexual impotence, warts, epilepsy, toothache, hemorrhoids, snake bites A poultice of the roots or stems is applied to nose ulceration, hemorrhoids, and swellings	Antimicrobial Antioxidant Antiviral Hepatoprotective	No report	13	[46]
	Uapaca kirkiana Mguhu (Bena)	Roots	Roots are boiled and the decoction is used as a remedy for indigestion and intestinal problems	No report	No report	02	[47]

(*Contd...*)

Table 1: (*Continued*)

Family	Botanical/common name	Part of the plant	Ethnobotanical preparation and use	Reported ethnopharmacology activity	Reported phytochemical profile or compound	Frequency of mention during FGD	Supporting literature
	Drypetes natalensis Hark	Leaves	Leaves decoction used against fever and malaria infections	Antitrypanosomal Antileishmanial	No report	01	[48]
	Croton macrostachyus Mulugu (Hehe) Liwurungu (Bena)	Leaves, stem and root barks	Stem barks decoction used for bathing babies against skin infections. Leaf decoction used against abdominal discomfort, sores and ring worms.	Antidiabetic Antimicrobial Purgative Anti-inflammatory Antiplasmodial	saponin, phenolic compound, tannins, anthocyanins, steroids, triterpens, alkaloids, coumarins, antraquinones, glucosides and essential oils	13	[49-52]
Fabaceae	*Dichrostachys cinerea* Mgegele/ mgegera (Hehe)	Leaves, stem and root barks	Grind the leaves and dress the wounds Roots decoction used for TB, infertility, venereal diseases, abdominal ulcers	Antidiarrheal Antibacterial Antioxidant Nephroprotective Immunostimulant	Terpenoids, tannins	07	[53-55]
	Albizia harveyi Msisina (Hehe)	Roots and leaves	Roots and leaves boiled then wash the affected parts and drink, fruits active for scabies, fungus and other skin diseases	Cytotoxic	Alkaloids, glycosides, saponins, Terpenes and flavanoids	11	[56,57]
	Cassia abbreviata Mulimuli (Hehe)	Roots, stem barks and leaves	Dry and powder the roots then take 1 tea spoon in water 3 times a day for strong fever, tooth ache, abdominal pains, back pains and feet pains	Antimicrobial Antimalaria Anti-HIV	Flavonoids, sterols, triterpenoids and anthraquinones	04	[58,59]
Hypoxidaceae	*Hypoxis hemerocallidea* Munyunyu (Hehe)	Roots-potato	Young tuber eaten fresh for diarheal, peptic ulcers and vomiting	Antimicrobial Antioxidant Anticancer Anti-HIV	Hypoxoside, rooperol, phytosterols, laectins, levoglucosan	01	[60,61]
Linaceae	*Hugonia castaneifolia* Ngaze (Hehe)	Root barks	Root barks used as a remedy against intestinal worms, malaria, fungus.	Antifungal Cytotoxic Larvicidal Antibacterial Antioxidant	Terpenoids, lignans	04	[62,63]
Loganiaceae	*Strychnos spinosa* Li/Mtangadasi (Hehe)	Leaves, stem barks and root barks	Sap from leaves used against snake bites	Acaricidal Antitrypanocidal Antimicrobial	Alkaloids, terpenoids, glycosides, flavonoids and tannins	12	[64-66]
Meliaceae	*Azadirachta indica* Mwarobaini (Kinga)	Leaves, stem and roots	Boil the roots and drink for treatment of syphilis	Antimicrobial	Tetranortriterpenoid, protolimonoid	15	[67,68]
Moraceae	*Ficus sycomorus* Mkuyu (Swahili)	Barks and whole plant	Barks powder used for body rashes	Antifungal Antibacterial Antioxidant Insecticidal Acaricidal	Quercetin, gallic acid, Rutin	05	[69-71]
Myrtaceae	*Eugenia capensis subsp. nyassensis* Kivengi/ Mkangaa (Hehe)	Roots	Powdered roots and sniff for -Head ache, flu and chest diseases	No report	No report	07	No report
Oleaceae	*Olax obtusifolia* De Wild Mtungapwezi	Roots	Leaves powder for treatment of pains	No report	No report	01	No report

(*Contd...*)

Table 1: (*Continued*)

Family	Botanical/common name	Part of the plant	Ethnobotanical preparation and use	Reported ethnopharmacology activity	Reported phytochemical profile or compound	Frequency of mention during FGD	Supporting literature
Rosaceae	*Prunus africana* Mwiluti (Hehe)	Roots	Boil the roots decoction and drink	Anti-inflammatory, Antispasmodic, Anticancer	Glycosides, terpenoids, sterols, fl-sitosterol, lauric acid, myristic acid, n-docosanol, ferulic	03	[72,73]
Rubiaceae	*Gardenia jovis-tonantis* Kilekamahame (Hehe)	Roots and leaves	Grind the roots make decoction drink and smelled for migraine Leaves for wounds	Cardio-relaxant Antisickling Antibacterial Antioxidant Antimalarial	Terpenoids, saponins,		[47,74,75]
	Breonadia salicina Ngwina (Bena)	Leaves, stem barks and roots	Roots decoction drunk as purgative Stem barks decoction for stomach-ache	Antimicrobial Antidiarrheal	No report	01	[76]
	Multidentia crassa Muwewe (Hehe)	Leaves and roots	Leaves are pounded, soaked in water and the juice applied into ears for ear infection Roots used for stomachache	No report	No report	11	Not reported
	Catunaregum spinosa Mpongolo (Hehe)	Roots barks, stem barks and leaves	Roots decoction for treatment of skin diseases, HIV, epilepsy, oral infection Grind the barks to make decoction and feed that child with convulsions. Its roots combined with *Dovyalis abyssinica* roots boiled and drink 3 times in 7 days for syphilis.	Cytotoxic Anthelmintic Antioxidant Sedative	Saponins, coumarins, Terpenoids, carbohydrates, glycosides, phytosterols, phenolic compounds, tannins and mucilage	08	[57,77-79]
Rutaceace	*Zanthoxylum chalybeum* Lungulungu (Hehe)	Leaves and roots	Drink the roots/leaves decoction-oral sores and ulcer	Antimicrobial	Isoquinoline alkaloids, protoberberines	18	[80,81]
	Toddalia asiatica Lutono (Hehe)	Leaves and roots	Leaves and roots decoction used for treatment of microbial diseases Hot infusion from barks for cancer and toothache	Antimalarial Anti-inflammatory Analgesic Sedative Antimicrobial Antioxidant Fungicide Inhibit HIV-reverse transcript tase	Flavanoids, alkaloids, tannins, steroids, phytosterols, saponins, glycosides, coumarins, carbohydrates coumarins, quinoline, nitidine	06	[82-84]
	Zanthoxylum deremense Engl Mkunungu-Hehe	Stem	A decoction of bark and roots is used as a remedy for malaria, generalized body pains, coughs, body swellings, anemia, and as a gargle for toothache Bark and root powder is mixed with oil and applied as liniment for pains and sprains Root bark is powdered and added to tea oral, two cups are taken twice daily	No report	No report	05	No report

(*Contd...*)

Table 1: (*Continued*)

Family	Botanical/common name	Part of the plant	Ethnobotanical preparation and use	Reported ethnopharmacology activity	Reported phytochemical profile or compound	Frequency of mention during FGD	Supporting literature
Santalaceae	*Osyris lanceolata* Mdunula (Hehe)	Stem and root barks	Stem barks decoction for treatment of Sexual Transmitted Diseases (STDs) A decoction of the bark and heartwood is used to anemia Leaves and roots used against backbones and stomach pains, fungus and typhoid	Antioxidant Antimicrobial Antifungal	Phenols, flavonoids sesquiterpenes and pentacyclic triterpenoids	17	[85-87]
Smilaceae	*Smilax anceps* (Mkwangasale)	Leaves	Leaves powder used for body rashes	Antimicrobial	Alkaloids and saponins	04	[88]
Solanaceae	*Solanum anguivii* Kumkalanga (Hehe)	Roots and fruits	Combined with leaves of Mkiringiti then use the decoction to wash the body Fruits chewed for coughs and chest pains	Antioxidant Antihypertensive	Saponins, phenols, flavanoids	14	[89,90]
	Solanum incanum Musufi/mtula (Hehe) Ndulele (Swahili)	Roots, leaves and fruits	Grind leaves and pressed the juice/ ointment over the affected tooth-teeth infections Fruits used for skin infections. Treatment of painful menstruation	Acaricidal effect Cytotoxic Anticancer Hypoglycemic Antimicrobial Antischistosomal Antinociceptive Antipyretic Antispasmolytic Anorexic	Anthraquinones, flavonoids, glycosides, carbohydrate and steroids	07	[21,64,91-94]

Table 2: Brine shrimp toxicity results of medicinal plants used in Southern Highland regions

Plant name	Part of plant	Solvent used	LC_{50} (μg/ml)	95% Confidence interval
Cyclophosphamide	NA	NA	16.3	10.6-25.1
Bidens pilosa	Leaves	Ethanol	107.15	69.94-164.15
Brachystegia spiciformis	Leaves	DCM	151.81	82.28-280.69
Cassia abbreviata Oliv.	Roots	Ethanol	140.89	108.21-183.44
Commiphora africana	Roots	Ethanol	122.04	75.28-197.84
Croton macrostachyus	Leaves	DCM	12.94	6.71-24.95
Diospyros usambarensis	Roots	Ethanol	>1000	-
		DCM	420.83	247.72-714.91
	Leaves	Ethanol	547.09	306.81-975. 46
Drypetes natalensis	Leaves	Ethanol	93	64.95-132.85
Eledendrum buchananii	Stem barks	DCM	>1000	-
Garcinia acutifolia	Leaves	Ethanol	54.18	25.16-46.44
Garcinia spp.	Stem barks	Ethanol	82.73	64.08-106.77
Hymenaea verrucosa	Stem barks	Ethanol	41.47	30.64-56.11
Kigelia africana	Roots	DCM	424	281.73-638.12
		Ethanol	557.92	315.52-986.35
	Stem barks	Ethanol	>1000	-
Lantana viburnoides	Stem barks	DCM	191.27	119.64-305.8
Leonotis lepetifolia	Leaves	Ethanol	>1000	-
Mucuna stans	Leaves	Ethanol	>1000	-
		DCM	488.05	281.63-845.79
Olax obtusifolia	Roots	Ethanol	77.09	60.15-98.81
Parinari curatellifolia	Stem barks	DCM	476.67	258.11-880.41
	Roots	Ethanol	>1000	-

(*Contd*...)

Table 2: (*Continued*)

Plant name	Part of plant	Solvent used	LC_{50} (μg/ml)	95% Confidence interval
	Leaves	Ethanol	175.05	119.61-256.2
		DCM	>1000	-
	Stem barks	Ethanol	>1000	-
	Roots	DCM	43.43	36.9-51.11
	Stem barks	Ethanol	>1000	-
Solanum incanum	Leaves	Pet. ether	>1000	-
Strychnos spinosa	Leaves	Ethanol	>1000	-
		Pet. ether	592.4	332.89-1054.24
		DCM	>1000	-
Terminalia sericea	Leaves	Ethanol	113.4	70.05-183.57
Zanthoxylum chalybeum	Roots	Ethanol	38.51	32.50-45.63
Zanthoxylum deremense	Stem barks	Ethanol	78.69	52.48-118

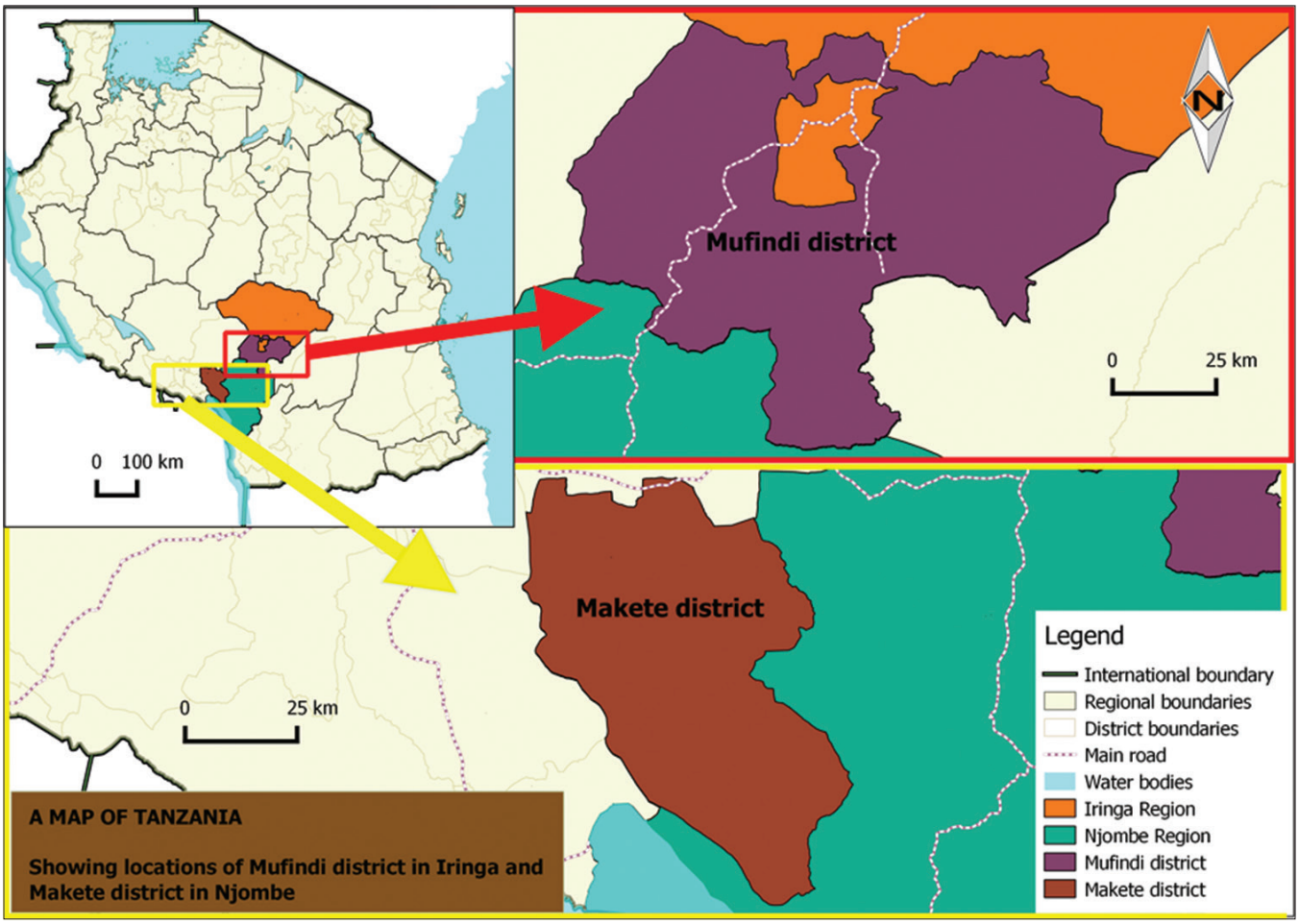

Figure 1: Map of Tanzania showing the study areas (Mufindi and Makete Districts) Ethnobotanical survey

cyclophosphamide (16.3 μg/ml). Other similar study undertaken on stem barks of this plant to evaluate cytotoxicity and acute toxicity in mice demonstrated the toxicity of the plant resulting in mortality of tested organisms [104]. The genus *Croton* has been reported to demonstrate moderate to high toxicities with proven the anticancer activity [51]. This knowledge triggers the use of plant products as complementary and alternative therapies both as direct and adjuvant remedy. A growing body of literature suggests the cancer preventive and therapeutic potential of phytochemicals and a lot of research has focused on the cellular mechanisms by which these phytochemicals interfere with the carcinogenic process. With the ability to target a variety of signaling pathways, phytochemicals are considered to be promising therapeutic agents against tumors with limited toxicity to normal cells.

CONCLUSION

The ethnobotanical survey has revealed that traditional health practitioners are rich in knowledge of fungal medicinal plants in these areas. These plants though have received little attention from modern biomedical research could be a promising source

of knowledge for the discovery of useful remedies if this wealth is preserved through proper documentation and research. Most of the plants collected were ascertained to be safe for use and hence could be considered for further scientific studies. The reported species may be used for the development of new, affordable, and effective herbal formulations for antifungal health-care management or used in drug discovery.

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