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Antifungal and Antibacterial Activity of Crude Stem Bark Extracts' of *Bersama abysinicca* Verdc. and *Faurea saligna* Harr.

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ABSTRACT

The two plant species were obtained from Mau Forest Complex in the Rift Valley, Kenya. Various parts of the two plants: *Bersama abysinicca* Verdc. Family, Melathiaceae and *Faurea saligna* Harr. Family, Proctaceae have been used in vogue by the Ogiek communities, who inhabit the area as their indigenous home, as source of traditional medicines for several ailments of human and livestock. *Bersama abysinicca* bark has been used in the management of blackwater east coast fever and rift valley fever in cattle. This study carried antimicrobial screening to uncover new antimicrobial agents from higher plants thus exposing their potentials. It is also aimed at demystifying the secrecy in which traditional African medicine has been shrouded for centuries. In the past, there has been no scientific verification to validate the efficacy of the traditional phytomedicine in the Ogiek community. Three methods were used to obtain various plant extracts. The first method was by obtaining crude plant extracts using methanol as a solvent. The second method was used by obtaining crude plant extracts through sequential extraction with ethyl acetate and petroleum ether as the third. Results showed that different plant species exhibited various bioactivities either on fungi, bacteria or on both categories of pathogens. The plant species extracts exhibited bactericidal and antifungal activities. Further researches should be carried out into the possibilities of formulation and commercialization of the plants as phytomedicine.

Key words: Livestock diseases, antimicrobial activities, sequential extraction, phytomedicine development

INTRODUCTION

Various parts of the two plants; *Bersama abysinicca* Verdc. (Fig. 1) (Melathiaceae) and *Faurea saligna* Harr. Family Proctaceae have been used in vogue by the Ogiek-researches as source of traditional medicines for several ailments of human and livestock.

Bersama abyssinica Fres. spp., *abyssinica* Verdc. Family Melanthiaceae (Sagawaita-Ogiek) researches on the, Ethnobotanical information, on the plants show that they are used for gastrointestinal conditions especially in Ivory Coast where reasonable efficacy against Gram negative and Gram positive bacteria were realized (Bolou *et al*., 2011). There were also evidences that the bark and leaves of the plant has antimalarial activities (Zirihi *et al*., 2010). The stem and leaves has been used in the management of blackwater east coast fever and rift valley fever in cattle by the Ogiek and Maasai alike (Towett, 2004). The same preparations have also been used against of malaria and fever in humans.

Fig. 1(a-b): *Bersama* (a) left, canopy plant, (b) fruits, Nessuit (April, 2007)

Fig. 2: *Faurea saligna* branch, Kaptuiget forest (Dec., 2007)

Faurea saligna, Harr. Proteaceae (Mosomboriet-Ogiek) (Fig. 2), the plants is used for several purposes in the sub Saharan, from ranging furniture to firewood and medicine. In Zimbabwe the studies reveal some levels of bioactivity of the plants crude methanol extracts (Chimponda and Mukanganyama, 2010). The plant concoctions are used by several communities within the Rift Valley of Kenya to treat various medical conditions which are bacteria and fungal related (Maara *et al*., 2014). The stembark has been used by the Ogiek community to sustain vitality in male human (Towett, 2004). The purpose of carrying out the antimicrobial screening was meant to uncover and verify the efficacy of the plants which are currently in use and to uncover new antimicrobial agents from higher plants thus exposing their potentials. It was also aimed at demystifying the secrecy in which traditional African medicine has been shrouded for centuries. In the past, there has been no scientific verification to validate the efficacy of the traditional phytomedicine in the Ogiek community.

The habit of the plant, *Bersama abyssinica* Fres. spp., *abyssinica* Verdc. Family Melanthiaceae (Sagawaita) (Voucher No. AO/025/NMK/15/04/2007) (Fig. 1) is: A Shrub or tree 1.5-24; bark smooth or rough, splitting lengthwise. Rachis of pinnate leaves round to broadly winged, in this specie, winged, the rachis wingless or only slightly winged capsule <2 cm long, globose, usually red-velvety;

leaves normally glabrous. Stamens 5; disc annul pentagonal 4-lobed; capsule often grooved. The species is endangered since only on tree remains in the whole of Mau Forest complex (Towett, 2004; Beentje, 1994).

About 1 kg of freshly collected bark cooked along with 5 ripe seeds of *S. eculeastrum* in 4 L of water. The resultant infusion, decanted and 1 L given daily for 3 days to animals suffering from black water, east coast fever and Rift Valley fever.

The plant *Faurea saligna* Harr. Procteaceae (Mosomboriet) (Voucher No. AO/021/NMK/04/12/2007), is characterized as: a tree about 20 m high, bark grey black deeply fissured. Leaves (narrowly) ovate to elliptic, base cuneate, apex acute, (5) 8-15 by 15-35 (45) cm glabrous or nearly so. Flowers cream with pink calyx in dense spikes, 6-14 cm long and corolla 15-18 mm long. Fruit small, round very hairy found in Afromontane forests. Endangered rare to find and only a few plants are found in Kaptuiget forest.

Scrapped fresh stem bark about 1 kg, boiled in 2 L of water cooled and 250 mL of resultant decoction taken daily as: Tonic to restore vitality in males, relive liver conditions and pneumonia.

MATERIALS AND METHODS

In the preparation of plant materials, there were two separate reconstitutions: Methanol extracts and sequential extractions. Specified portions of the individual plants were collected and dried at room temperature under shade till all the moisture was finished, milled to pass through a sieve of 0.50 mm diameter. The powder, of each sample, was hermetically sealed in polythene bags and stored till the time of use, if not extracted immediately.

Methanol extraction: The plant powders were individually extracted with methanol while fresh leaves were steam distilled. Some powdered material (50 g) were weighed into conical flasks (250 mL), covered with aluminium foil and then filled with about 200 mL of methanol. This was allowed to stand for 24 h.

Sequential extraction: The plants, *F. saligna* and *B. abysinicca* were further separately, sequentially, extracted with methanol, petroleum ether and ethyl acetate. The extracts were *F. saligna* a reddish powder and *B. abysnicca* a yellowish gel. Each of the extracts was put into individual vials tightly corked, labeled and stored in darkness in 4°C for future use.

Reconstitution of the test samples: The 0.1 g of each individual dried material was dissolved in about four Drops of Dimethyl Sulphoxide (DMSO), so as to make it absorbable by the test organisms and topped up to 1 mL of water.

Screening for antimicrobial activity: Antimicrobial efficacies were tested using the filter paper disc diffusion method (Elgayyar *et al*., 2001). A solution of each was prepared by dissolving 200 mg in 1 mL of methanol and 10 mL of the solution were dispensed onto 6 mm sterile filter paper discs and dried (2 mg $disc^{-1}$). The Muller-Hinton and Potato Dextrose Agar (PDA) were used in the culture of bacteria and fungi, respectively.

Each figure was seeded with 0.1 mL of bacterial and yeast culture directly from the 24 h broth culture diluted to match 0.5 and 1.0 Mac Farlands standard, respectively (10^8 Colony Forming Units (CFU mL^{-1}) and fungi diluted to match 1.0 Mac Farland standard (10^8 spores mL^{-1}).

Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC): Known weights of individual test plant extracts which had previously shown bioactivity during the bioassay tests were weighed out into sterile test tubes and then reconstituted with four droplets of Dimethyl Sulphoxide just to dissolve it and then topped with sterile distilled water to the required volume of 10 mL. Further, serial dilutions were made to give a total multiple of four concentrations 10, 20, 30 and 40 μm L^{-1}, respectively.

RESULTS AND DISCUSSIONS

Results for the various tests of the crude plants' methanol extracts: Table 1 shows a general view of plant species that act on bacteria and fungi. The + sign was used to indicate any sensitivity to the drugs. A single + indicated 2 mm inhibition from the edge of the disc of 6 mm in diameter, ++ indicated moderate activity +++ high activity and - indicating no reactions.

The organisms were further subjected to the same tests but at increasing concentrations that exhibited increase in the areas of inhibitions which varied from one organism to the other as can be seen in the figures thereafter, the subsequent contain individual extracts efficacies with quantum concentrations against different organisms including their sub cultures. Some of the sub cultures are universally known resistant to current broad spectrum antibiotics. The plants crude methanol extracts showed good antimicrobial activities on them.

There was resistance of fungi against the majority of the plant extracts with a few showing activities. This could be attributed to the fact that the drugs are able to penetrate the organisms' cell wall. The inability may be, due to the fact that fungi being plants, though from the lower classes may have similar compounds as the extracts. *Scleria biflora* was found to be effective on *K. pneumoniae, S. aureus, P. aeruginosa* and *E. coli*. *Faurea saligna* was effective on *E. coli*, *S. typhi* and *E. faecalis* while *B. abyssinica* was effective on *K. pneumoniae*, *S. aureus*, *E. coli* and *C. albicans* (Table 1) with StDev = 0,456 which was significantly different. There were variable activities of individual methanol extracts from one species to the other.

The following strains of *Pseudomonas* were used and all had similar reactions. *Pseudomonas aeruginosa*, *P. spp*, *P. spp* (KEMRI collection) *P. aeruginosa* ATCC27853, *P. aeruginosa* (hospital strain). All the strains responded similarly to the treatments by extracts from both the plant species) (Table 2).

Test organisms used are: *Candida albicans* (clinical isolate) *Candida brusei*, *Cryptoccus neoformas*, *Trichophyton mentagrophytes* (clinical isolates), *Microsporum gypseum* (clinical isolate) (Table 4) *Candida albicans* (clinical isolate), (Cb) *Candida brusei*, (Crn.) *Cryptoccus neoformas*, (T.m) *Trichophyton mentagrophytes* (clinical isolates), *Microsporum gypseum* (clinical isolates. Leaves distillate of the plant showed higher dosage as compared to others against *T. mentagropytes*. The lowest activity was obtained from *T. mentagrophyte* implying that this extract could possibly be formulated into medicine.

In Table 4 it was clear that there was activity across all the pathogens with MeOH root extracts but with highest potency from root bark petroleum ether extracts. This signified that most of the active principles are non polar soluble and are, likely to be, deposited in the roots. However, ethyl acetate, a weaker solvent, had the least activity from the root bark extracts.

Table 1: Screening for activity

Plants	S.a	P.a	E.f	K.p	E.c	S.t	C.a	C.b	C.n	T.m	M.g
Faurea saligna	+++	++	-	++	-	-	+++	+++	+++	++	+++
Bersama abysinicca	+++	-	+	+++	-	-	++	+++	++	+++	++

+++: Highly active, ++: Active, +: Slightly active, -: No activity, S.a: *Staphylococcus aureus*, C.a: *Candida albicans*, P.a: *Pseudomonas aeruginosa*, C.b: *Candida brusei*, K.p: *Klebsiella pneumoniae*, C.n: *Cryptococcus neoformas*, E.c: *Escherichia coli*, T.m: *Trichophyton mentagrophyte*, S.t: *Salmonella typhi*, M.g: *Microsporum gypseum*

Table 2: Zones of inhibition in (mm) Methanol extracts of *Bersama abyssinica* and *Faurea saligna* against strains of bacteria, species of *K. pneumoniae, P. aeruginosa* and *S. aureus*

	Zones of inhibition *B. abyssinica*					Zones of inhibition *F. saligna*			
Pathogens	a	b	c	d	Pathogens	a	b	c	d
Klebsiella pneumoniae	10	15	15	20	*Klebsiella .pneumonia*	8	10	10	12
Klebsiella oxytoca	9	15	16	20	*Klebsiella pneumoniae* (hospital strain)	8	10	10	12
Klebsiella pneumoniae (hospital strains)	11	17	17	21	*Klebsiella oxytoca*	8	10	10	12
Klebsiella pneumoniae (MDRS)	10	15	16	19	*Klebsiella pneumoniae* (Belgium strain)	8	10	10	12
Klebsiella pneumoniae (WHO)	10	15	16	19	*Klebsiella pneumoniae* (WHO std)	8	10	10	12
Klebsiella pneumoniae (Belgium strains	10	16	16	20	*Klebsiella pneumoniae* (MDRS)	8	10	10	12
Staphylococcus aureus (hospital strain)	15	14	14	16	*Staphylococcus aureus* (hospital strain)	8	9	12	16
Staphylococcus aureus (oxford strain)	15	15	14	16	*Staphylococcus aureus* (oxford strain)	8	9	12	16
Staphylococcus aureus (β haemolytic)	15	15	15	14	*Staphylococcus aureus* (β haemolytic)	8	9	12	16
Staphylococcus aureus (Danida strain)	15	14	15	15	*Staphylococcus aureus* (Danida strain)	8	9	12	16
Staphylococcus aureus (Pigmented with staphylokinase)	15	15	15	15	*Staphylococcus aureus* (Pigmented with staphylokinase)	8	9	12	16
Staphylococcus aureus (ATCC 20591)	15	14	15	15	*Staphylococcus aureus* (ATCC 20591)	8	9	12	16
Staphylococcus. aureus (ORSA) and MRSA	15	14	14	15	*Staphylococcus aureus* (ORSA) and MRSA	8	9	12	16
Pseudomonas aeruginosa	10	10	10	10	*Pseudomonas aeruginosa*	10	12	14	16

a: Extract conc. 20 mL mL^{-1}, b: Extract conc. 40 mL mL^{-1}, c: Extract conc. 80 mL mL^{-1} and d: Extract conc. 160 mL mL^{-1}

There are explanations that *Salmonella typhi* and *Pseudomonas aeruginosa* are Gram negative bacteria, having complex protein in the cell wall, such that it is not easily penetrable by certain molecules. Extracts from, *Faurea saligna* and *Bersama abyssinica* were effective against most of the bacteria and *Candida albicans*. However, none of the extracts were effective against *Cladosprium* spp. and *Trichophyton* spp. *Scleria biflora* was found to be effective on *K. pneumoniae*, *S. aureus*, *P. aeruginosa* and *E. coli*, *F. saligna* was effective on *E. coli*, *S. typhi* and *E. faecalis* while *B. abyssinica* was effective on *K. pneumoniae*, *S. aureus*, *E. coli* and *C. albicans*.

Bersama abyssinica stem bark extracts showed significant effect of inhibition against *K. pneumoniae* at various levels of conc. The $p<0.05$ suggesting that these results confirm the ethnobotanical surveys whereby the plant is used for various ailments in human like fever rheumatism and east coast fever. Although the extract was active against *S. aureus*, there was not much difference in response to in livestock increase in concentrations in the case of *Klebsiella* spp. The $p<0.05$ in the case *S. aureus*, *B. abyssinica* is not mentioned in other literature as a medicinal plant of significance. However, in East Africa several communities use it to treat colds, aphrodiciac, purgative emetic and anti diarrhoea (Kokwaro, 1993). The records on its efficacy and boiactivities are limited in literature. The information on efficacy on growth inhibition at 50% at concentration 0.08 g mL^{-1}, against *K. pneumoniae* (Fig. 3) justifies its use as a remedy for colds and chest related complaints by the community in the study. The experience of inhibition was the same in case of *S. aureus* and its strains.

Faurea saligna gave results which indicated a specific activity on *P. aeruginosa* strains, in that it was the only single plant species whose extract gave an ever-increasing zone of inhibition proportionally to the increase in concentration without any deviation. This scenario was the same for both *S. aureus* and *K. pneumoniae*. Apart from the concentrations in other species of the pathogens, the same concentrations there were no reactions in all the strains and species of *E. coli*, *E. faecalis* and *S. typhi*.

In the past three decades, traditional medicine has been accepted as an alternative form of healthcare (WHO., 1978). The development of microbial resistance to the available antibiotics has led several people to investigate the antimicrobial activities of higher medicinal plants. Bisignano *et al*. (1996), Maoz and Neeman (1998) and Homer *et al*. (2000). There is also increased

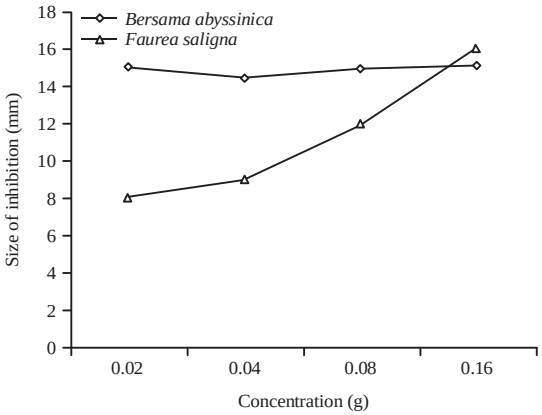

Fig. 3: Stem bark MeoH extra inhibition of *Klebsiella* spp

use of plants extracts as cosmetics and pharmaceutical products, something which has generated a lot of interest in identification of active compounds. In doing so, it is imperative to carry out systematic studies on plants. This therefore means that, plants being sessile are capable of synthesizing a vast array of secondary metabolites as defence mechanisms for protecting themselves against pathogenic infections. Despite the fact that there are several natural and synthetic products available to ameliorate fungal and bacterial maladies, it is recognized that both resistant fungi and bacteria are on the increase (Wang and Huang, 2005; Wang *et al*., 2006). Consequently, there is a need to detect new sources of antifungal compounds with potential application in medicine and also as additives in food and food preservatives.

There have been reports of systematic biological investigations of the *in vitro* antimicrobial activities of alcohol extracts of some plant extracts from all over the world that are used in traditional medicine (Queiroz *et al*., 2004). The exploration of new antimicrobial compounds has led to collaborative interests in natural products chemistry, agriculture and medicine in fundamental research. Isolation and identification of compounds which have antibiotic effects without being cytotoxic to cells of higher plants and animals could be beneficial. A serious indicator of activity is to establish the minimum concentration of the bioactive crude extracts of the plant that could inhibit microbial growth and the concentration that could kill the microbes completely referred to as Minimal Bactericidal Concentration (MBC).

Virtually, all the antibiotics that are currently in use in allopathic medicine are subject to challenges in that they are subject to effective resistances by pathogenic microorganisms to the extent that they are rendered ineffective within a short duration of their introduction into the regimen. However, one way of ensuring their efficacy is by carrying out studies to determine their MIC, MBC and MFC's. It is rather unfortunate that such tests are often done *in vitro* visa vie *in vivo*. In cases where laboratory animals are used, such tests are not necessarily the same as in humans and the other higher mammals.

To date the term antibiotics often includes synthetically produced antibacterial and anti-fungal substances. The first major classes of antibiotics were introduced in the 1940s and 1950s. In bacterial infections they were hailed as miracle drugs that eliminated bacteria without doing much harm to cells of the treated individuals (Sade *et al*., 2003). Soon there was a glut of varieties of the drugs being introduced into the market which became saturated. Many pharmaceutical firms have since then shied away from the development of new antibiotics and have instead focused on

Table 3: MIC and MBC tests with methanol extracts from the two pant species

Plant	Organism	MIC	MBC
B. abysinicca	*Pseudomonas* spp.	500	1000
	Klebsiella spp including MDRS	1000	2000
	Staphylococcus aureus including ORSA	50	50
F. saligna	*Pseudomonas* spp.	25	1000
	Staphylococcus aureus including (ORSA)	<25	2000
	K. pneumoniae	<25	<25

F. saligna showed better inhibitory activities as compared to *B. abysinicca*, MIC: Minimum inhibitory concentration, MBC: Minimum bacterial concentration

Table 4: Antifungal activity of various plant parts extracts of *B. abysinicca*

	Test fungi				
Plant parts and extract	C.a	C.k	Cr.n	T.m	Mg.
Stem bark					
Methanol	5.50	5.25	4.00	11.5	17.00
Petroleum ether	2.75	4.50	3.00	15.0	5.75
Ethyl acetate	-	-	-	-	20.00
Leaves					
Methanol	-	-	-	-	-
Petroleum ether	-	-	-	-	-
Ethyl acetate	-	-	-	-	-
Root bark					
Methanol	4.00	6.00	3.75	12.5	5.50
Petroleum ether	1.25	3.00	2.50	10.0	4.50
Ethyl acetate	-	-	-	-	-

C.a: *Candida albicans*, Cr.n: *Candida brusei* T.m: *Trichophyton mentagrophyte*, M.g: *Microsporum gypseum*

combating chronic diseases (Barnes, 2003). The problem of antibiotic resistance is aggravated by two factors. One is overuse of antibiotics both in humans and animals and second non-compliance of patients to the courses of treatment. Both the long-term exposure to low doses and the failure to finish a prescription encourage more resistant bacterial strains to thrive (Homer *et al*., 2000). The universal problem of pathogen resistances to antibiotics is compounded by the emergence of opportunistic infections both fungal and bacterial infections (Seguin *et al*., 2006). The infectious diseases such as fungal dermatitis have become more frequent due to immuno compromised and immuno-suppressed conditions arising from HIV conditions. The management of such cases is more often resistant to known antimycotic drug than before (Normark and Normark, 2002).

The results obtained were reflective of the information gathered in the field in that certain extracts exhibited both bacteriostatic and bacteriocidal activities, for example as in the case of *B. abysinicca* inhibited the growth of *S. aureus* at 50 μg mL^{-1} and could not allow any growth even after the sub culture and further incubation giving a similar MBC (Table 3).

Sequential extractions of stem barks of *B. abysinicca* and *F. saligna* showed varied fungicidal activities at various concentrations as could be seen in the Table 4. The laboratory value given in the graph below would otherwise demonstrate the organism as being bacteriostatic to contain the organism as the body builds its immune systems (Aguilar, 2001).

Extracts from *Bersama abysinicca* and *Faurea saligna* showed activities against both Gram negatives like *Pseudomonas aeruginosa.* The case was the same when tested against all the other strains including hospital drug resistant ones at an MIC of 500 and at 100 μg mL^{-1}. *Klebsiella pneumoniae* including MDR strains had MIC of 100 μmg mL^{-1} and MBC of 200 μg mL^{-1}. The *S. aureus* including ORSA had MIC and MBC at 50 μmg, indicating that the extract was active against this organism. These studies concur with uses to which the community put the plant products at the local levels. However, the plants of the Melanthiaceae family are mentioned in

cases involving cardiovascular pharmacopoeia of biological origin (Houghton, 2002). Both the root and the stem methanol and petroleum ether extract possessed activities against all the test organisms with having the degree of freedom (df ≥2) at a concentration of 2000 μg mL^{-1}. This is a good indication of the plant being a high potential for drug development. The African species have not been fully explored to ascertain their ability to provide scientific base for pharmaceutical development.

It was recorded during the ethnobotanical survey that the bark of *Faurea saligna* is used by the community as aphrodiasic and tonic. Its ethanol extract was active against three organisms tested, that is, *Pseudomonas, Klebsiella* and *Staphylococcus* and the MIC and MBC for these organisms and their strains were less than 25 μg mL^{-1}. This indicates a high level of potency against Gram-ve bacteria and common bacteria like *Staphylococcus* which develop resistance rather fast and often (Mallorqui-Fernandez *et al*., 2004). Although there is research potential in this plant very little or no work has been done on its pharmacognosy. Its activity against *P. aeruginosa* and its other strains makes it a good potential for research since this organism is responsible for nosocomial infections (Guillemot *et al*., 2005). Because of the nature of certain metabolites to respond to solvents differently, it became necessary to use conventional extractive solvents. This in the end yielded levels of activities, as shown in the Table 3 and 4 MIC/MBC and MFC.

Furthermore, sequential extractions of various portions of the plant had significant activities against selected human pathogenic fungi. When various exracts were set against the organisms with flocunazole as control, the ANOVA for all the extracts from both parts of the plant showed df ≥2 with leading results in the Petroleum Ether followed by methanol. Although, ethyl acetate was not active against most of the test organisms, it was significantly active against *Microsporum gypseum* with a test of df≥ 3 when compared to other extracts. Outstanding activities across the board were also realized when dealing with the extracts from the root bark. The phenomena are that most of the metabolites are transported from the sites of synthesis and ultimately stored in the roots in majority of the higher plants. This is perhaps why the activity is higher in root extracts.

RECOMMENDATIONS

Major thrust by whole of the pharmaceutical industry is currently paying attention towards design and development of new novel and indigenous plant based products. This is being achieved through investigations that are relying on leads from traditional system of medicine; it is therefore recommended that an interdisciplinary approach be employed in studying the community to achieve the aforementioned goal.

It is recommended that there should be a proper epidemiological record keeping so as establish the efficacy of the drugs used in traditional systems on endemic diseases and the newly emerging and reemerging ones. This would ensure that alternative ways are evolved to combat the current world problems of multi drug resistances of microbes to the currently known antibiotics.

For purposes of scientific progressiveness, phytochemists should be carrying out comprehensive elucidation of the active principles so that pharmacists may work on the several combinations and cocktails which are effective and could be used in the manufacture of new drugs to combat the maladies currently afflicting humankind. Although several plants were identified as being able to provide remedies to several health complaints, but did not give positive results. It does not imply that they are ineffective. Further researches should be carried out using animal models under laboratory conditions to ascertain their efficacy and validate their continued use for the complaints that the traditional healers claim they alleviate. Using the same drugs and techniques

further studies should be carried out on similar organisms to ascertain their broad spectrum efficacies. This could lead to discovery of new drugs for the management of such cases as cancers, various viral and fungal infections which are on the increase but are unfortunately unmanageable with the current drugs in the market.

Sequential extraction methods should be carried out on all the drug plants in order to reveal and identify those compounds which are less polar as compared to the methanol extracts. This method could reveal what is implicit those plants with higher efficacies that could be used in the discovery of new drugs.

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