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MULTI-PLANT OR SINGLE-PLANT EXTRACTS, WHICH IS THE MOST EFFECTIVE FOR LOCAL HEALING IN TANZANIA?

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Abstract

Use of multi-plant extracts against infectious diseases is increasing in rural Tanzania. The study evaluated this ethnomedicinal practice by using mixed root extracts of *Carisa edulis, Ximenia caffra, Harrisonia abyssinica* and *Euclea natalensis* against single extracts of the same plants. Disc diffusion assay and Tube dilution techniques were used to compare bioactivity of plant extracts *in-vitro.* The ANOVA test indicated significant difference ($P < 0.05$) between these extracts types. Multi-plant extracts had inhibition zones of up to 26mm as compared to 14mm for single extracts. Minimum Inhibitory Concentration for multi-plant extract was 8.3µg/ml against 69µg/ml for single extracts. Multi-plant extracts inhibited all the five test bacterial species while single extracts inhibited three species. Eight out of ten multi-plant extracts (80%) were bactericidal while only two out of four single extracts (50%) were bactericidal. Generally, multi-plant extracts were more superior over single plant extracts and could be developed into more potent antibiotics against resistant pathogens.

Key words: Bioactivity, ethnomedicinal practices, mixed plant extracts, single plant extracts, Tanzania, traditional healers

Introduction

The use of multi-plant extracts in traditional healing systems is a common ethnomedicinal practice in many parts of the world. In Venda (Cyprus), mixed root extracts of *Tabenaemontana elegans, Terminalia sericea, Euclea natalensis, Ximenia caffra and Ziziphus mucronata* are used to treat sterility (Arnold and Gulumian, 1984). In Malawi in Africa, the root decoction of *Xylopia parviflora, Heteromorpha arborescense* and *Carissa edulis* is drunk to increase penile size and for treating loss of libido in males (Msothi and Mgombo, 1993). In Tanzania, the use of multi-plant extracts is believed to cure complicated infections as compared to single plant extracts. In rural areas of Lake Victoria region in Tanzania, elephant dung is believed to treat gastrointestinal and opportunistic infections in infants basing on the fact that the beast consume a variety of plants with the possibility that some may have medicinal value that are then egested in active form for medicine. Likewise, fresh chyme extracted from the gut of

slaughtered healthy goat is used to relieve constipations and gastrointestinal problems in humans based on the same principle as elephant dung.

The dialogue with local people in Lake Victoria basin in Tanzania revealed that use of multi-plant extracts in their area has increased in recent years due to emergence of secondary opportunistic infections related to HIV/AIDS. This view is in compliance with WHO (2004) report that, resistance of bacteria to antibiotics is made even more complicated because of adverse reactions exhibited by the hosts, especially by HIV positive patients. Such resistance hinders traditional management of infections by using single plant extracts. Apart from microbes' resistance, high costs and inadequate services in conventional health system in Tanzania also contribute to high attendance of patients to traditional healers. It is estimated that Tanzania has over 75,000 traditional healers attending over 80% of the rural communities as compared to about 1,500 registered medical doctors (Moshi and Mbwambo, 2004). This indicates that traditional healers contribute immensely in primary health care and hence the need to conduct research into their herbal remedies based on the method used by the healers to establish their effectiveness, either as multi-plant or single-plant extracts.

Although there are diverse combinations of plant extracts, very common multi-plant regimen used by many traditional healers in Tanzania and specifically in Lake Victoria region includes root decoction of *Carisa edulis* (Forssk.) Vahl (Apocinaceae), *Ximenia caffra* Sond (Olacaceae), *Harrisonia abyssinica* Oliv (Simaroubaceae) and *Euclea natalensis* L. (Ebenaceae) for the treatment of gonorrhea, syphilis, skin infections, eruptions on the skin, boils, internal swellings, chronic amoebic dysentery and typhoid fever. The use of multi-plant and single-plant extracts in traditional healing system has also been reported by Mahunnah et al. (2006) on their work in Eastern Tanzania. The researchers observed that out of the 1297 plant preparations (formulas) recorded for different uses; most remedies consisted of single plants. However, 5.5% consisted of simple therapeutic combinations of two to seven plants.

Despite the common use of multi-plant extract therapies by traditional healers in Tanzania, there is no scientific evaluation report on superiority of this ethnomedicinal practice over single plant extracts. This paper presents results of disc diffusion bioassays subjected to both the mixed and single plant extracts against the standard laboratory microbes. In this study, the terms "multi-plant extracts" and "plant extract mixtures" are used interchangeably.

Methodology

Description of the study site

The information on the use of multi-plant extracts was obtained from traditional healers in Lake Victoria basin in Tanzania. The area was selected due to the predominant use of multi-plant concoctions to treat venereal and opportunistic infections.

Data collection

Ethnobotanical information on the use of medicinal plants was collected by the research team using semi-structured questionnaires (in Swahili language) between March and December, 2006 after prior signed consent protocols with traditional healers. Traditional healers (33 female and 23 male) were identified objectively at the village government offices depending on their conceived competence by the fellow villagers. A checklist of medicinal plants and their preparations was established based on information from traditional healers, and this comprised both multi-plant and single plant recipes. The most common plant extract mixture consisted of *Carissa edulis, Euclea natalensis, Harrisonia abyssinica* and *Ximenia caffra*. Root samples of these four plants were collected in Lake Victoria basin by one of the authors (Otieno, J.N.) and then authenticated by Prof. R.L.A. Mahunnah, a taxonomist at the Institute of Traditional Medicine, Muhimbili University of Health Sciences and Allied Sciences (MUHAS). Voucher specimens were deposited at the Herbarium of the Institute of Traditional Medicine, MUCHS, under the accession numbers: Otieno 0031 for *C. edulis, E. natalensis* (Otieno 0020), *H. abyssinica* (Otieno 0012) and *X. caffra* (Otieno 0011).

The extraction of plant specimens was according to Draper (1976). Plant materials (root barks) from Tarime District in Lake Victoria, Tanzania were shade dried at room temperature and in well ventilated room for two weeks and then plant materials were ground separately to a 60-mesh powder at the Department of Chemistry-University of Dar es Salaam. The powder, approximately 600g for each of the

test plants, was separately extracted in 1000ml of 95% ethanol using a soxhlet apparatus for 48hrs. The extracts obtained were concentrated in *vacuo* at 40°C using a rotary evaporator. The extraction resulted into 24g reddish crystals of *X. caffra*, sticky and light brown extract (12.3g) from *C.edulis*, sticky and dark brown 22.5g extract from *H. abyssinica* and 18g black extract from the roots powder of *E. natalensis*.

Preparation of single and multi-extract mixtures

There were four single root extracts prepared from each of *C. edulis*, *E. natalensis*, *H. abyssinica* and *X. caffra*. The remaining eleven extracts were root mixtures consisting of two, three and four plant extracts randomly mixed from the four extract of the same plant species. Table 2 presents the mixing procedure in which there is individual plant extracts (no. 1-4) and mixtures of ethanol root extracts. All extracts were regarded as independent treatment regardless on whether they were singles or mixed extracts.

Test micro-organisms

All 15 treatments or root extracts in Table 2 were tested against gram negative bacteria namely *Escherichia coli* (DSM 1103), *Pseudomonas aeruginosa* (DSM 1117) and *Salmonella typhi* (NCTC 8385). Also against the following gram positive bacteria; *Staphylococcus aureus* (ATCC 25923), *Klebsiella pneumoniae* (NCTC 9633) and local strains of *Streptococcus faecalis* (authenticated at the Department of Microbiology and Immunology, Muhimbili University College of Health Sciences, Tanzania). Micro-organisms were obtained from the laboratory of Molecular and Biotechnology Department, University of Dar es Salaam, Department of Microbiology and Immunology, Muhimbili University College of Health Sciences, and Faculty of Veterinary Medicine, Sokoine University of Agriculture, Tanzania. The test organisms for this study were selected because they are the common pathogenic clinical isolates in the laboratories. *Escherichia coli* is one of the common causes of neonatal meningitis, intestinal and urinary tract infections (Ryan, 1984a). The genus *Streptococci* includes pathogens of human that causes variety of acute infections such as rheumatic fever, acute glomerulonephritis, neonatal meningeal infection, bacterial pneumonia etc (Ryan, 1984b). *Staphylococcus* colonizes the arterial nares and sometimes other skin sites of about 30% of people in the community. The species is pathogenic and causes variety of epidermal and sub-cutaneous infections such as pyogenic, suppurative lesion, boils, bullous impetigo etc. *S. aureus* can cause variety of deep tissue infections including osteomyelitis, arthritis, cerebral, pulmonary and renal abscesses. The species can also cause pneumonia and wound infection, food poisoning and various toxic substances (Sherris, 1984). Some of these diseases are managed by traditional healers by using the mixed extracts of the decoctions of the selected plants in this study.

Culture media

The medium used for growing bacteria was Nutrient Agar (NA) and Nutrient Broth (NB); both were of analytical grade, OXOID. The media were prepared according to manufacturer' specifications. Monthly sub-culturing of the pure strains from stock culture slants was routinely done at the Molecular and Biotechnology Department, University of Dar es salaam (UDSM) so as to maintain the pure strains.

Bioactivity testing technique
Disc diffusion assays

Antimicrobial testing began with disc diffusion assay according to Platt (1986), followed by more sensitive tube dilution technique. The aim of disc diffusion assay was to test if both mixed and single plant extract categories were active against the test microorganisms. During the disc diffusion assay, plant extracts 10µl volume per disc from the stock solutions of $10mgml^{-1}$ of each of 15 treatments were impregnated in standard filter paper discs of 7mm diameters by using a micropipette. Petri dishes containing nutrient agar media were flooded with 0.2 ml of the test inoculum (10^5 c.f.u) then spread by Grigalsky spatula. The plates were desiccated 5-10 mins for adequate drying at room temperature in a sterile lamina flow. The paper discs impregnated with plant extracts were then placed on inoculated plates, and then left at least for 6 hrs at 4^0C to allow diffusion of extract into the agar before microbial cells started to multiply. Standard antibiotic (Ampicillin 25 µg) and an organic solvent dimethylsulfoxide (DMSO) were used as positive and negative controls respectively. The plates were then incubated at 37 ± 1^0C and the zones of inhibition (mm

diameter) were measured after 24 hrs. Bioactivities of the antimicrobial agents were indicated by clear zones of inhibition including the area covered by the disc, and these zones of inhibitions were measured in millimeters (mm) by a transparent ruler.

Tube Dilution method

Tube Dilution method according to the standard protocol by Carter and Chengappa (1991) was used to determine minimum active concentrations of the plant extracts that showed positive results during disc diffusion assays. In tube dilution method, plant extracts were diluted two-fold in a series of ten tubes. Nutrient broth 1.8ml was added into the test tube one, and 1ml of nutrient broth was added to the second, third up to the tenth test tube. Each test tube with nutrient broth was inoculated with appropriate seeded nutrient broth (10^5 c.f.u ml^{-1}) that was prepared before. In different activity, plant extract samples were diluted in dymethylsulfoxide to obtain a stock solution of $10mgml^{-1}$. The plant extract sample 0.2 ml from the stock solution was added to tube one with 1.8ml of the seeded nutrient broth to form the first dilution of $1.1mgml^{-1}$. One milliliter of this solution was transferred into tube two to give second dilution of 556µg/ml, each time 1ml of a solution from a preceding tube was transferred into the next tube until the most dilute concentration of 2µg/ml was formed in the last tube. The eleventh tube contained seeded broth without plant extract and the twelfth tube was not inoculated to determine the level of contamination. The eleventh and twelfth tubes were controls. There were triplicate sets of serial dilutions for each plant extract. A set of tubes containing seeded broth and suitable solvent controls were incubated at 37 ∓ 1^0C and the MICs (μgml^{-1}) of products (based upon visual appearance of growth) recorded after 24h post-incubation. The last seeded tube with no apparent growth of the organism was taken to represent the MIC of the test compound.

Minimum Bactericidal Concentration (MBC)

One drop of 10^5 (c.f.u/ml) of the inoculum for each microorganism was introduced in each of the series of 10 test tubes as explained in tube dilution method. Plants extract samples 0.2ml was added into first to tenth test tubes. Eleventh test tube was a control as it was not treated with plant extracts. Before incubation, 0.2ml of culture solution was pipetted off from the drug free or control tube and subsequently spread onto the surface of sterile agar medium using a sterile glass Drigalsky spatula. This was done for each of 15 plant extracts. The plates and test tubes were incubated at 37^0C with the test tubes being in the shaking incubator. After 24 hrs of incubation, 0.2ml of solution from each of the test tubes showing no growth was sub cultured on agar media plates and incubated at 37^0C for 24 hrs. The resulting bacterial colony numbers on the plates were compared to those on the plates from the control tubes. Then the MBC were recorded in mg/ml or µg/ml. In this process, when the number of colonies observed were similar to that from the control before incubation, that became the indication of bacteriostatic action. However, when there was no growth in the culture plate, this indicated complete killing or bactericidal.

Data analysis

The analysis of variance compared variances in bioactivity of treatment means of bioactivity of multi-extract concoctions against single extracts. The null hypothesis was that there was no difference in bioactivity of multi-plant extracts to individual plant extracts, while the alternate hypothesis was that bioactivity differed.

Results

The study revealed that, although most common diseases in rural areas of Lake Victoria region-Tanzania are locally managed by single plant extracts, mixtures of more than one plant extract are often used to treat complicated diseases. Out of 83 health problems recorded in Lake Victoria region in Tanzania, eleven diseases were recorded as being managed by using mixtures of two or more plant extracts (Table 1). The mixed root extracts of *E. natalensis, C. edulis, X .caffra* and *H. abyssinica* were the most frequently mentioned multi-plant extract for various diseases. Use of these plants in different combinations was cited by 26 traditional healers out of all the 56 traditional healers interviewed.

Table 2 shows 15 different plant extract treatments (both single and multiple extracts) derived from the root extracts of *E. natalensis, C. edulis, X. caffra* and *H. abyssinica*. The extracts number 1 to 4 is single extracts of each of the source plant, while extracts 5 to 15 are mixture of two to four plant extracts. Bioactivities of the plant extracts against the test microbes are presented in Table 3. The values in Table 3 are the diameters in millimeters of zones of inhibition by plant extracts against the test microbes, Minimum Inhibition Concentrations (MIC) of plant extracts and Minimum Bacterial Concentrations (MBC) for test microorganisms. Values for the bioactivities of plant extracts are presented by diameters of circles around paper disc in millimeter including areas occupied by discs. MIC and MBC of different plant extracts were taken for the most susceptible microorganisms to specific plant extracts only. The most susceptible microorganisms were characterized by largest zone of inhibition.

Table 1: List of different health problems treated with multi-plant extracts in Lake Victoria region, Tanzania

	Disease		**Plant extract mixtures**
1	Yellow fever	i.	1+2
		ii.	7+22+31+43
		iii.	1+16+17+20
2	Venereal diseases	i.	14+15
		ii.	5+14+34+35
		iii.	5+7+14+28
3	Opportunistic infections	i.	1+14+7+16+17+18+19+20+21+22+23+24+25+26+27
		ii.	7+22+31+43
		iii.	5+14+25+47+48+49
		iv.	5+7+14+25+27+35+37
		v.	5+7+14+15+22+25
		vi	5+7+14+28
4	Infertile women		5+26+40
5	Swollen pancreas		12+13
6	Amoebic dysentery	i.	8+10+9+11+50
		ii.	51+52
		iii.	5+7+14+28
7	Boils		33+44
8	Pneumonia		45+46
9	Nervous discomfort of feet		4+39+40+41+42
10	Measles		17+38
11	Bilharziasis		4+5+6+7

Key; 1. *Mangifera indica* 2. *Senna sp* 3. *Cajanus cajan* 4. *Sclerocarya birrea* 5. *Harrisonia abyssinica* 6. *Acacia hockii* 7. *Carissa edulis* 8. *Cissus rotundifolia* 9. *Vernonia sp* 10. *Boscia angustifolia* 11. *Kigelia africana* 12. *Commiphora africana* 13. *Spermacoce chaetocephala* 14. *Euclea natalensis* 15. *Zanthoxylum chalybeum* 16. *Psidium guajava* 17. *Penisetum purpureum* 18. *Cymbopogon citratus* 19. *Punica granatum* 20. *Musa sp* 21. *Kedrostis foetidissima* 22. *Withania somnifera* 23. *Acacia robusta* 24. *Eucalyptus sp* 25. *Ximenia caffra* 26. *Clerodendrum mrycoides.* 27. *Dichrostachys cinerea* 28.*Ximenia caffra* 29. *Gomphrena celosioides* 30. *Cardiospermum halicacabum* 31. *Piliostigma thonningii* 32. *Maytenus senegalensis* 33. *Gynandropsis gynandra* 34. *Gomphocarpus fructicosus* 35. *Aloe secundiflora* 36. *Ricinus communis* 37. *Acacia brevispica* 38. *Cassia grantii* 39. *Rytigynia oligacanta.* 40. *Rhoicissus revoillii.* 41. *Acalypha ornata* 42. *Melhania velutina* 43. *Salvadora persica* 44. *Achyranthes aspera* 45. *Ozoroa mucronata* 46. *Grewia bicolor* 47. *Capparis fascicularis* 48. *Sansevieria enhlenbergia* 49. *Asparasgus flagellaris* 50. *Cyphostemma orondo* 51. *Hoslundia opposita* 52. *Ocimum basilicum*

Table 2: Different 15 root extract mixtures derived from the most common multi-plant regimen in Lake Victoria region, Tanzania

Extract no.	Formulation/mixture
1	*Euclea natalensis*,
2	*Carissa edulis*
3	*Ximenia caffra*
4	*Harrisonia abyssinica*
5	*Euclea natalensis, + Carissa edulis*
6	*Euclea natalensis + Ximenia caffra*
7	*Euclea natalensis + Harrisonia abyssinica*
8	*Carissa edulis + Ximenia caffra*
9	*Carissa edulis + Harrisonia abyssinica*
10	*Ximenia caffra + Harrisonia abyssinica*
11	*Euclea natalensis +Carissa edulis+ Ximenia caffra*
12	*Euclea natalensis+ Ximenia caffra+ Harrisonia abyssinica*
13	*Carissa edulis+Ximenia caffra+Harrisonia abyssinica*
14	*Euclea natalensis+ Carissa edulis + Harrisonia abyssinica*
15	*Euclea natalensis +Carissa edulis+ Ximenia caffra+ Harrisonia abyssinica*

Table 3: Single and Multi-extract activity of four plants on pathogenic bacteria

Extract no.	***S.typhi*** (mm)	***S. aureus*** (mm)	***S. faecalis*** (mm)	***E.coli*** (mm)	***P. aeruginosa*** (mm)	**MIC** µg/ml	**MBC** mg/ml
1	0	14	0	0	0	55±4.4	0
2	0	0	18.5	18	0	55±3.3	1.1
3	13	0	19	0	0	55±4.5	0
4	23	12	12	0	19	17±2.3	0.017
5	11	15	0	0	0	55±2.6	0
6	11	0	0	15	20	34±4.4	0
7	18	12	12	0	20	17±5.4	0.335
8	11	0	0	0	16	34±2.3	0
9	21	10	11	0	20	17±1.3	0.335
10	11	13	0	0	20	17±2.1	0.335
11	11.5	0	0	18	17	17±0.6	0.017
12	17.5	17	0	0	22	17	0.017
13	15.5	13	10	0	18.8	17±1.3	0.017
14	20	11	11	0	21	17±0.9	0.017
15	20	15	15	18	22	8.3±0.6	0.017

Key: MIC = Minimum Inhibitory Concentration, MBC = Minimum Bactericidal Concentration

Of all 15 treatments, three multi-plant and two single extracts were bacteriostatic namely, *X. caffra* and *E. natalensis* against *P. aureginosa; E. natalensis* and *C. edulis* against *S. typhi*; *C. edulis* and *X. caffra* against *S. typhi; E. natalensis* against *P. euruginosa* and *X. caffra* against *S. typhi* respectively. The remaining ten extracts including two single and eight multi-plant extracts were bactericidal.

Both single and multi-plant extracts in this study were effective against the test bacteria. However, the result of the analysis of variance ($P < 0.05$) implied that mixing of plant extracts significantly improved bioactivities of multi-plant extracts over individual plant extracts. Table 3 shows that the bioactivities of multi-plant extracts were comparatively superior to single plant extracts. The mixture of four extracts had inhibition zones ranging from 18 to 22mm, whereas bioactivity of a single plant extract of *E. natalensis* had the least inhibition of 14mm. With the exception of *H. abyssinica*, single plant extracts had narrow activities or were active to one to three species of bacteria only, while multi-plant extracts inhibited 3 to 5 test bacteria (Table 3). Mixing of the extracts of different plants improved bioactivities of original inactive

ingredients. This is shown in Table 3 that extracts of *E. natalensis* and that of *C. edulis* were originally inactive against *Salmonella typhi,* however, when mixed, they were in-turn active to the same bacteria. Mixing of plant extracts for the case of this study also improved bioactivity of crude plant extracts against resistant microbes. *Escherichia coli* in this experiment revealed broad resistance to individual extracts. Similar test for antibacterial activity in Guinea Conakry found that ethanol extract (95%) of the root extract of *H. abyssinica* at 1mg/ml is inactive against *E.coli* (Balde et al., 1995). Methanol extract of *X. caffra* root in Kenya have weak activity against *E. coli* at 2.0mg/l **(**Fabry et al., 1998), but in this experiment, *E.coli* growth was inhibited by a mixture of four extracts. Multi-extract regimen in this study was able to inhibit the growth of *P. aeruginosa* that was reported by Walker and Edwards (1999) to be naturally resistant to most antibacterial agents. Therefore, multi-plant extracts not only had higher bioactivity, but also had broad activity to large number of bacteria strains. For all extract constituents, *H. abyssinica* had higher bioactivity even more than some multi-plant extract. This high bioactivity of *H. abyssinica* contributed more to the activity of other plant extracts in the mixture. According to Table 2, all top most bioactive mixtures contained *H. abyssinica*.

Discussion

The results in this study support preference and effectiveness of mixed extracts by traditional healers in management of opportunistic infections. From the literature, some of these infections are caused by pathogenic i.e. *Staphylococcus* species that causes epidermal and sub-cutaneous infections, lesion, boils, cerebral, and abscesses that are some of secondary opportunistic infections. *Salmonella* species causes gastrointestinal problems, and *Streptococcus* species causes some respiratory infections in HIV patients (Centers for Disease Control and Prevention, 2002). The test microbe *Staphylococcus, Streptococcus* and *Salmonella* species in this study were readily inhibited by most of mixed extracts *viz*. extracts no. 10, 12, 13, 14 and 15 in Table 3. Mixed extracts of the selected test plants also inhibited other microbes that cause diseases not mentioned by traditional healers in the study area. This significant potency of extract mixtures affirms broader bioactivity against larger number of pathogenic microbes.

In traditional healing schemes where there is no prior laboratory tests to diagnose the exact causes or agents for the ailments, use of multi-plant extract regimens expand the confidence level that, most of the causes of the ailment may be managed with any one of the mixture's ingredients. The practice of dispensing multi-plant regimen by traditional healers is a unique indigenous knowledge that starts as a trial and error, though later experienced traditional healers develop standard dosages for each of the extract ingredients in conformity to the conventional pharmaceutical protocols**.** The unit measurement of each plant extract by traditional healers in Lake Victoria was a "finger pinch" of a dried and powdered root, though the number of finger pinches varied with traditional healers and the ailment. One draw of a finger pinch was estimated to 0.5-2mg in a standard tea cup. Despite the benefits of multi-plant extract regimens, the scheme is incongruent to ethical considerations that inhibit clinical testing of plant extract with unknown health hazards to human beings. There was no formalized safety protocol for mixing of different plant extracts. Each traditional healer had unique combination and dosage that was directly dispensed to patients. Most traditional healers in Lake Victoria basin, Tanzania served 3 cups of a multi-plant regimen per day for amoebic dysentery, while some served up to a liter in 24 hrs. The highest dosage of the multi-extract was for venereal diseases in which up to 6 tea cups of the multi-extract was being served per day. The anticipated risk for using multi-plant extract regimens may be the same as extract-drug combination as commented by Williams (2001) that potential interaction of herbal medicines with drugs is a major safety concern, especially, for drugs with narrow therapeutic indices (e.g. warfarin and digoxin), and may lead to adverse reactions that are sometimes life-threatening. Coupled with the above, there are so diverse formulations for a particular ailment (Table 1) such as opportunistic skin infections and yellow fever that make it cumbersome to select a direction for drug development. Nevertheless, in all extract mixtures for various diseases as in Table 1, there were key or common species for each disease category. For example, *Carissa edulis* appeared five times in each of six mixtures for opportunistic skin infections. Likewise, *Mangifera indica* appeared more frequently for the mixtures against yellow fever. The plant species that appears most frequently in different mixtures for a particular disease suggests the respective species to be a prospective candidate for drug development for that particular disease.

Conclusion and recommendations

The mixture of root extracts from four plants namely *E. natalensis, C. edulis, X. caffra* and *H. abyssinica* in Tanzania had higher bioactivity than individual extracts of the same plants. Multi-plant extracts not only had higher bioactivity, but also had broad activity against large number of bacteria including some naturally resistant microbes. Despite the relevance of multi-extract therapy, traditional healers should be assisted to standardize their regimens by carrying out toxicological tests for safe dispensing. The mixture of plant extracts in this study may be developed further into standard antibiotics for a broader application against diverse types of resistant pathogenic microbes.

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