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Antimicrobial and cytotoxicity activity of selected Pare folk medicinal plants

Bazı Pale yöresi şifalı bitkilerinin antimikrobiyal ve sitotoksik aktivitesi

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ABSTRACT
Aim: The study aimed to evaluate antimicrobial and cytotoxicity of *Grewia villosa*, *Cassia abbreviata* and *Hoslundia opposita*.
Methods: Microdilution method and brine shrimp lethality test were employed to evaluate antimicrobial and cytotoxicity of plant extracts.
Results: The extracts of *G. villosa*, *C. abbreviata* and *H. opposita* showed different levels of antimicrobial activities on Gram negative bacterial and fungal species tested. The minimum inhibition concentration (MIC) ranges of *G. villosa*, *C. abbreviata* and *H. opposita* extracts were 0.391-25, 0.195-25 and 0.391-25 mg/mL respectively. *Cassia abbreviata* stem methanolic extracts and roots ethyl acetate extracts exhibited the highest activity with minimum inhibition concentration (MIC) of 0.195 mg/mL against *S. typhi* and *K. oxytoca* respectively. At least one of the extract from each plant exhibited a cytotoxicity nature when tested on brine shrimps where the *G. villosa* stem chloroform extract was the most cytotoxic with fifty percent lethal concentration (LC50) of 48.432 µg/mL.
Conclusion: The findings in this study validate the traditional use of *G. villosa*, *C. abbreviata* and *H. opposita* in management of bacterial and fungal infections. Further phytochemical investigations on *G. villosa*, *C. abbreviata* and *H. opposita* growing in Pare Mountains are recommended to contribute to drug development.
Keywords: *G. villosa*; *C. abbreviata*; *H. opposita*; Pare Mountains.

ÖZET
Amaç: Çalışmanın amacı, *Grewia villosa*, *Cassia abbreviata* ve *Hoslundia opposita* bitkilerinin antimikrobiyal ve sitotoksik aktivitesinin incelenmesidir.
Yöntemler: Bitki ekstraktlarının sitotoksik ve antimikrobiyal aktivitesi mikrodilüsyon ve brine shrimp lethality test ile incelendi.
Bulgular: *G. villosa*, *C. abbreviata* ve *H. opposita* ekstraktları mantar ve Gram negatif bakteri türlerine farklı düzeylerde antimikrobiyal aktivite gösterdi. *G. villosa*, *C. abbreviata* ve *H. opposita* ekstraktlarının minimum inhibisyon konsantrasyonları (MIC) sırasıyla 0,391-25, 0,195-25 ve 0,391-25 mg/mL idi. *Cassia abbreviata* gövdesinin metanolik ve kökünün etil asetat ekstraktı *S. typhi* ve *K. oxytoca* ile kıyaslandığında 0,195 mg/mL MIC değeri ile en yüksek aktiviteye sahipti. Her bitkinin en az bir ekstraktı brine shrimps ile test edildiğinde sitotoksik aktivite gösterdi. *G. villosa* gövdesinin kloroform ekstraktı 48.432 µg/mL %50 letal konsantrasyonu ile en yüksek sitotoksik aktiviye sahipti.
Sonuç: Bulgular, *G. villosa*, *C. abbreviata* ve *H. opposita*'nın bakteriyel ve fungal enfeksiyonlardaki geleneksel kullanımını doğruladı. Pare dağlarındaki *G. villosa*, *C. abbreviata* ve *H. opposita* ile ilgili daha fazla çalışma ilaç gelişimtirme çalışmaları açısından tavsiye edilir.
Anahtar kelimeler: *G. villosa*; *C. abbreviata*; *H. opposita*; Pare Dağları.

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INTRODUCTION

Pathogenic bacteria and fungi have been a great causative agents of many diseases affecting both human and livestock. They cause diseases such as gastrointestinal infections, food poisoning, flu/cold, coughing, pneumonia, tuberculosis, candidiasis and other skin diseases to mention a few. These diseases are currently treated by synthetic antibiotics developed from drug templates of plant and microorganisms origin [1, 2]. Despite of their

effectiveness, some of the antibiotics are being challenged by antibiotic resistance from the allied microbes [3]. In order to address resistance problem, researches have been searching for novel drug templates with unique mechanism of actions of which medicinal plants offers a promising source [4-7].

Ethnomedical information has therefore been utilized as stepping stone towards unveiling drug templates from the medicinal plants [4, 6, 7]. The present study therefore focused on validating the ethnomedical use of *Grewia villosa, Cassia abbreviata* and *Hoslundia opposita* used by Pare communities in Tanzania as a remedy for microbial infections. These plants are also utilized in other communities in Africa. For instance, *G. villosa* is used for the treatment of wounds, syphilis, eye infections, gastrointestinal infections, boils, tuberculosis, gonorrhea, urinary tract infections, cholera and diarrhea [8-11]. *Cassia abbreviata* is widely used for management of skin diseases, cough, pneumonia, fever and gonorrhea [12-15]. *Hoslundia opposita* is used treatment of gonorrhea, cystitis, wounds, sores, conjunctivitis and gastrointestinal diseases [16-18]. This paper therefore reports the antibacterial, antifungal and cytotoxicity activities of thirty (30) extracts from *Grewia villosa, Cassia abbreviata* and *Hoslundia opposita*.

METHODS AND MATERIAL

Chemicals and reagents

Chloroform was purchased from Lobal chemie Laboratory Reagents and Fine chemicals, India ethyl acetate and methanol were procured from RFCL Ltd, Haryand, India and Avantor Performance Material India LTD respectively. Absolute ethanol was procured from Scharlab S. L. Spain and Diethyl sulphoxide (DMSO) was obtained from Avantor Performance Material India LTD. Fluconazole was acquired from Lincoin Pharmaceuticals LTD, India, Ciproflaxine were bought from Micro Lab LTD, India and Cyclophosphamide was bought from Khandelwa Laboratories Pvt Ltd (Mumbai), Iodonitrotetrazolium chloride (INT) was purchased from SIGMA (Sigma Aldrich, St Louis, USA). Nutrient agar and broth, Sabouraud dextrose agar and broth were all purchased from Hi Media Laboratories Pvt Ltd (Mumbai-India).

Test organisms

The microorganisms involved in this study were sub cultured *Klebsiella oxytoca* (clinical isolate), *Klebsiella pneumoniae* (ATCC700603), *Salmonella kisarawe* (clinical isolate), *Proteus mirabilis* (NCTC 1075), *Salmonella typhi* (NCTC 8385), *Pseudomonas aeruginosa* (ATCC 29953) and *Escherichia coli* (ATCC 25922). The fungi species used in the assay include *Cryptococcus neuformans* (clinical isolate) and *Candida albicans* (ATCC90028). For brine shrimp lethality test, nauplii (brine shrimps larvae) were used.

Plant materials

The leaves, twigs, stem and root parts of *Grewia villosa, Cassia abbreviata* and *Hoslundia opposita* were collected from their natural environment in Kisiwani ward, Same district, Kilimanjaro-Tanzania. Identification was done by Mr. Emmanuel Mboya a botanist from Tropical Pesticides Research Institute (TPRI) and the voucher specimens coded GVEL - 12, CAEL – 22 and HOEL - 16 for *Grewia villosa, Cassia abbreviata* and *Hoslundia opposita* respectively were deposited at Nelson Mandela African Institution of Science and Technology.

Preparation of plant extracts

The chopped stem and root barks and leaves were spread on the flour and left to dry at room temperature ranging from 27ºC to 30ºC. They were then pulverised into fine powders. The pulverised plant materials (500 g each plant part) were consecutively soaked in chloroform, ethyl acetate and methanol for 48 hours. The extracts were filtered through a Whatman No. 1 filter paper, and then concentrated *in vacuo* using Rotary evaporator. All extracts were stored in refrigerator at -20°C until the testing time.

Anti-microbial screening

Minimum Inhibitory Concentrations (MICs) of plant extracts against the tested organisms were determined by micro dilution method [19] using 96-well microtitre plates. The plates were first preloaded with 50 µL of the nutrient broth for bacteria tests and sabouraud dextrose broth for fungal tests. Then it was followed by an addition of 50 µL of the extracts 100 mg/mL (prepared in DMSO) into the first wells of each row to make a total volume of 100 µL in the first wells. After thorough mixing 50 µL were drawn from each of the first row wells and put into the subsequent rows to the last wells where the drawn 50 µL was discarded. Thereafter, 50 µL of the bacterial suspension (0.5 Mac Farhland standard turbidity- a suspension containing about 5×107 CFU mL-1) was then added in each well to make the final volume of 100 µL. Ciproflaxine were as standard drugs for bacteria and Fluconazole was as standard drug for fungi. DMSO was used as a solvent control and nutrient broth and

sabouraud dextrose broth were used as negative control for bacteria and fungi respectively. The plates were then incubated at 37°C for 24 h. For each extract, MICs were determined by adding 10 µL of 0.02% p-iodonitrotetrazolium (INT) chloride dye in each well followed by incubation for 1 h at 37°C. Microorganism growth was indicated by a change of colour from purple to pink indicating active growth. The lowest concentration which showed no bacterial or fungal growth was considered as Minimum Inhibitor Concentration (MIC). MIC values were interpreted as follows 0.05-0.5 mg/mL strong activity, 0.6-1.5 mg/mL moderate activity and above 1.5 mg/mL weak activity [20].

Brine Shrimps Lethality Test

The artificial sea water of 3.8 g/L was prepared using sea salt to provide a suitable environment for hatching brine shrimps eggs [21]. The glass container was used as a hatching container and was partition into two compartments with a piece of holed dull glass in between. The smaller compartment was covered by a black paper while the other one was left to be illuminated. The prepared sea water was poured in a glass container, then eggs were spread on dark compartment of a container and the light was illuminated on other sides of larger illuminated compartment. The light will attract hatched shrimps to move onto illuminated side through holes in a separating glass placed in between compartments. The shrimps took 48 hours to hatch into larva. Afterward the nauplii (brine shrimps larva) were picked by Pasteur pipette from the lightened side into beaker with artificial sea water. Then ten brine shrimp larvae were added in into each universal bottle containing small amount of prepared sea water followed by filling the bottled to make a total volume of 5 mL. Extract solutions prepared at concentrations of 8, 24, 40, 80, 120, 240 µg/mL were added to the universal bottles. The same procedure was for the standard (cyclophosphamide) and negative control which was the solvent used to dissolve extracts. After 24 h the surviving nauplii were counted and the Lethal Concentration (LC) values of extracts were calculated at 95% Confidence Interval and the concentration for 100% mortality were calculated using regression equation for each extract.

Data analysis

The Microsoft Excel 2010 computer software was used to obtain regression equation, and from which LC_{50}, LC_{16}, and LC_{84} were calculated. The 95% Confidence Interval was then calculated using method reported in [22]. The results were used to document safety and cytotoxicity activity of plant extracts. The LC_{50} greater than 100 µg/mL was considered non-toxic and below it as toxic [23].

RESULTS

Antimicrobial activity

All tested extracts exhibited antimicrobial activity against *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Salmonella kisarawe*, *Proteus mirabilis*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Candida albicans* and *Cryptococcus neuformans* with varied potencies as indicated by the minimum inhibition concentration (MIC) in Table 1. The MIC range of *G. villosa*, *C. abbreviata* and *H. opposita* extracts against Gram negative bacteria were 0.391-25.000, 0.195-25.000 and 0.391-25.000 mg/mL while against fungal species were 0.781-25.000, 0.781-25.000 and 0.781->25.000 mg/mL respectively. Based on the MIC range, *C. albicans* was more resistant to plant extracts with MIC range of 1.563->25.000 mg/mL as compared to *C. neuformans* which exhibited MIC range of 0.781-25.000 mg/mL.

Hoslundia opposita leaf chloroform, *Grewia villosa* root methanol and *C. abbreviata* stem methanol roots ethyl acetate and methanol extracts exhibited antibacterial activity against Gram negative bacteria with a narrow MIC ranges of 0.391 - 1.563, 0.391 - 3.125, 0.195 - 1.563, 0.195 - 3.125 and 0.781 - 1.563 mg/mL respectively. Furthermore extracts that exhibited strong antibacterial activity were *C. abbreviata* stem and roots methanol extracts with MIC ranges of 0.195 -1.563 mg/mL and 0.781 – 1.563 mg/mL respectively. However, it has been observed that the tested fungal species were relatively resistant to the tested plant extracts except for *C. abbreviata* leaf chloroform and methanol extracts which had a MIC value range of 0.781 – 1.563 mg/mL.

Table 1. Antimicrobial activities of *G. villosa*, *C. abbreviata* and *H. opposita* extracts.

Extract type	Minimum Inhibition Concentration (MIC) mg/mL								
	Microorganisms								
	K. oxytoca	***K.pneumonia***	***P. euriginosa***	***S. kisarawe***	***S. typhi***	***P. mirabilis***	***E. coli***	***C. neuforma***	***C. albicans***
GVLC	12.500	12.500	6.250	6.250	12.500	6.250	12.500	6.250	12.500
GVLE	6.250	6.250	3.125	3.125	12.500	3.125	6.250	3.125	12.500
GVLM	3.125	0.781	3.125	1.563	3.125	6.250	0.781	1.563	3.125
GVSC	25.000	25.000	6.250	6.250	6.250	6.250	6.250	6.250	12.500
GVSE	0.391	6.250	3.125	6.250	6.250	3.125	3.125	6.250	12.500
GVSM	12.500	12.500	12.500	3.125	0.781	12.500	1.563	3.125	6.250
GVRC	25.000	25.000	6.250	25.000	6.250	12.500	12.500	6.250	25.000
GVRE	0.781	12.500	6.250	6.250	6.250	12.500	12.500	6.250	25.000
GVRM	3.125	1.563	3.125	0.391	0.781	1.563	1.563	0.781	3.125
CALC	3.125	3.125	3.125	3.125	3.125	1.563	1.563	0.781	1.563
CALE	12.500	12.500	6.250	25.000	6.250	12.500	12.500	12.500	6.250
CALM	3.125	3.125	3.125	3.125	6.250	6.250	3.125	0.781	1.563
CATC	6.250	6.250	6.250	12.500	6.250	12.500	12.500	12.500	25.000
CATE	3.125	6.250	3.125	6.250	3.125	6.250	6.250	6.250	3.125
CATM	12.500	>25.000	12.500	12.500	6.250	12.500	12.500	6.250	12.500
CASC	25.000	25.000	6.250	3.125	6.250	12.500	25.000	6.250	12.500
CASE	3.125	3.125	0.781	6.250	0.781	3.125	3.125	6.250	12.500
CASM	1.563	0.781	1.563	1.563	0.195	0.391	0.781	6.250	6.250
CARC	12.500	12.500	25.000	6.250	12.500	12.500	25.000	6.250	6.250
CARE	0.195	1.563	1.563	3.125	0.391	0.781	0.781	6.250	3.125
CARM	1.563	1.563	1.563	1.563	0.781	0.781	1.563	6.250	12.500
HOLC	0.391	0.391	1.563	0.391	1.563	1.563	1.563	6.250	12.500
HOLE	3.125	12.500	3.125	0.781	3.125	6.250	12.500	0.781	3.125
HOLM	3.125	12.500	6.250	12.500	6.250	6.250	25.000	3.125	6.250
HOSC	6.250	3.125	3.125	6.250	3.125	6.250	6.250	25.000	12.500
HOSE	6.250	12.500	12.500	12.500	0.781	12.500	12.500	6.250	12.500
HOSM	1.563	12.500	6.250	12.500	6.250	6.250	25.000	1.563	3.125
HORC	6.250	6.250	3.125	6.250	6.250	3.125	6.250	12.500	6.250
HORE	6.250	12.500	12.500	12.500	12.500	12.500	12.500	6.250	>25.000
HORM	1.563	3.125	1.563	1.563	0.781	0.781	3.125	6.250	12.500
Cipro	0.781	0.391	0.391	0.781	0.391	0.781	0.391	NA	NA
Fluco	NA	NA	NA	NA	NA	NA	NA	>25.000	>25.000

KEY: GVLC = *Grewia villosa* leaves chloroform extract GVLE = *Grewia villosa* leaves ethyl acetate extract, GVLM = *Grewia villosa* leaves methanol extract, GVSC = *Grewia villosa* stem chloroform extract, GVSE = *Grewia villosa* stem ethyl acetate extract, GVSM = *Grewia villosa* stem methanol extract, GVRC = *Grewia villosa* roots chloroform extract, GVRE = *Grewia villosa* roots ethyl acetate extract, GVRM = *Grewia villosa* roots methanol extract, CALC = *Cassia abbreviata* leaves chloroform extract, CALE = *Cassia abbreviata* leaves ethyl acetate extract, CALM = *Cassia abbreviata* leaves methanol extract, CATC = *Cassia abbreviata* twigs chloroform extract, CATE = *Cassia abbreviata* twigs ethyl acetate extract, CATM = *Cassia abbreviata* twigs methanol extract, CASC = *Cassia abbreviata* stem chloroform extract, CASE = *Cassia abbreviata* stem ethyl acetate extract, CASM = *Cassia abbreviata* stem methanol extract, CARC = *Cassia abbreviate* root chloroform extract, CARE= *Cassia abbreviate* root ethyl acetate extract, CARM = *Cassia abbreviata* roots methanol extract, HOLC = *Hoslundia opposita* leaves chloroform extract, HOLE = *Hoslundia opposita* leaves ethyl acetate, HOLM = *Hoslundia opposita* leaves methanol extracts HOSC = *Hoslundia opposita* stem chloroform extract, HOSE = *Hoslundia opposita* stem ethyl acetate extract, HOSM = *Hoslundia opposita* stem methanol, HORC = *Hoslundia opposita* roots chloroform extract, HORE = *Hoslundia opposita* roots ethyl acetate extract, HORM = *Hoslundia opposita* roots methanol extract, Cipro= Ciproflaxine, Fluco = Fluconazole.

Cytotoxicity activity

Grewia villosa, *C. abbreviata* and *H. opposita* extracts were evaluated for lethality activity against the brine shrimp larvae and results are summarized in Table 2. The interpretation of the results was according to criteria developed by Moshi *et al*., (2010) in which LC_{50} value < 100 µg/mL was considered toxic and LC_{50} value > 100 µg/mL nontoxic. It is evident that out of thirty extracts evaluated, nine extracts representing 30% of all extracts had LC_{50} value < 100 µg/mL. The level of cytotoxicity between plant species varied with *H. opposita* being the most toxic (66.67%) followed by *G. villosa* (22.22%) and *C. abbreviata* (11.11%). *Grewia villosa* stem chloroform extract was the most cytotoxic with LC_{50} 48.432 µg/mL but it was less cytotoxic by the factor of 3 compared to the cyclophosphamide. With exception of *G. villosa* root methanolic and *H. opposita* leaf ethyl acetate extracts, the rest of extracts which exhibited high antimicrobial activity had LC_{50} greater than 100 µg/mL implying low toxicity to brine shrimp larvae. It was however interesting to observe that *C. abbreviata* leaf chloroform extract which inhibited the growth of *C. neuformans* at MIC value of 0.781 mg/mL had no toxicity against brine shrimp larvae with LC_{50} 687.837 µg/mL.

DISCUSSION

Hoslundia opposita chloroform leaf extract were found to be relatively active on the tested bacteria highlighting the presences of antibacterial nonpolar compounds. Previous phytochemical study on *Hoslundia opposita* growing in Nigeria, Ivory Coast and Cameroon revealed the presence of monoterpenes [24-26] and germacrene [27] which suggest that antibacterial activity exhibited by *Hoslundia opposita* chloroform leaf extract might be due to terpenes. The *C. abbreviata* methanol and chloroform leaf extracts exhibited antifungal activity against *C. neuforma* highlighting potential for antifungal drug discovery. Furthermore, leaves from *C. abbreviata* growing in Northern Botswana have been reported to contain alkaloids, tannins, anthraquinones, flavonoids and polyphenols [12, 14, 28]. Likewise, leaves from *Grewia villosa* growing in Saudi Arabia was established to contain high concentration of alkaloids and flavonoids [29, 30] and further proved to possess antimicrobial properties [31, 32]. Presence of these compounds in *Grewia villosa* growing in Pare Mountains might contribute to the observed antimicrobial activities.

Leaves extracts from *G. villosa* and *C. abbreviata* were relatively low cytotoxic when tested in the brine shrimp lethality test as none of them displayed 50% lethal concentration (LC_{50}) below 100 µg/mL. This suggests that the leaves of these plants are both safe toward human cells and efficient as antimicrobial agents against Gram negative bacteria. The use of leaves is highly recommended for sustainability of the plant as the use of roots and stems increases risk of plant extinction should there be over harvest of roots and stems. *Hoslundia opposita* ethyl acetate and methanol leaf extracts exhibited high cytotoxic against brine shrimp larvae with LC_{50} value below 100 µg/mL suggesting to contain secondary metabolites which are potential antitumor agents. Since *H. opposita* leaf extracts had weak antimicrobial activity against the tested bacterial and fungal species, the focus for succeeding studies is recommended to be on the unveiling antitumor drug templates.

The *C. abbreviata* stem methanol and ethyl acetate extracts had relative high antibacterial activity supporting the ethnomedical uses of the plant [10, 12, 14, 33]. *Cassia abbreviata* growing in Zambia was reported to inhibit the growth of *Staphylococcus aureus*, *Shigella Spp*., *Bacilus Spp*. and *Proteus Spp*. which corroborate with the present findings. *Cassia abbreviata*, *H. opposita* and *G. villosa* stem bark extracts have unveiled antimicrobial properties [1, 4, 26, 34] which supports the ethnomedical use of these plants. The tested *Salmonella spp* and *E. coli* are known for their importance in food poisoning, diarrhoea and other stomach/ gastrointestinal abnormalities [35, 36]. Therefore the recorded high activity of methanol root extracts of *G. villosa* supports its traditional use in stomach pain as reported in [2, 5]. They also had good antifungal activity against *C. neuforma*. Presence of alkaloids reported in [37] further strenghthen the observed activity. Good antibacterial activity that was recorded from the ethyl acetate and methanol extracts of *C. abbreviata* roots supports their traditional uses [10, 14, 15, 38]. These roots barks are also used as treatment for oral and vaginal candidiasis [12], supported by the antifungal activity displayed by ethyl acetate root extract against C. *albicans* in this study. The *C. abbreviata* roots are known to contain among other compounds polyphenols, anthocyanin, anthropoids, anthraquinones and tannins [10] which may have contribution on their activity.

Table 2. Brine shrimp activity of Grewia villosa, Cassia abbreviate and Hoslundia opposita extracts.

Extract type	Regression equation	LC50 (µg/mL)	95% Confidence interval (CI)	Regression coefficient (r)
GVLC	y = 63.042logx - 93.822	191.147	139.633 - 261.661	0.918
GVLE	y = 53.049logx - 89.377	423.961	96.923 - 1854.405	0.925
GVLM	y = 37.897logx - 41.366	257.575	32.637 - 2032.782	0.896
GVSC	y = 64.107logx - 58.029	48.432	35.5802 - 65.868	0.978
GVSE	y = 65.273logx - 108.72	270.168	199.489 - 365.889	0.956
GVSM	y = 85.975logx - 126.68	113.505	90.198 - 142.435	0.917
GVRC	y = 45.484logx - 47.587	139.805	90.489 - 215.999	0.984
GVRE	y = 85.54logx - 164.75	323.982	257.128 - 408.217	0.983
GVRM	y = 55.609logx - 49.58	61.761	43.25 - 88.195	0.972
CALC	y = 40.981logx - 66.283	687.837	424.074 - 1112.94	0.978
CALE	y = 59.683logx - 109.91	477.884	343.061 -665.692	0.889
CALM	y = 57.778logx - 83.794	206.850	146.806 - 291.452	0.994
CATC	y = 39.946logx - 43.038	213.351	130.013 - 350.109	0.984
CATE	y = 39.193logx - 71.454	1255.653	760.081 - 2074.339	0.825
CATM	y = 48.446logx - 68.543	279.840	170.530 - 459.217	0.978
CASC	y = 22.279logx - 43.917	16424.585	6753.520 - 39944.591	0.867
CASE	y = 57.011logx - 76.55	165.862	117.217 – 234.695	0.955
CASM	y = 46.1logx - 50.159	148.815	96.885 - 228.58	0.957
CARC	y = 22.279logx - 43.917	16424.585	6753.520 - 39944.591	0.867
CARE	y = 56.552logx - 53.692	68.166	48.038 - 96.72	0.996
CARM	y = 46.936logx - 70.957	377.626	247.624 - 575.88	0.967
HOLC	y = 88.139logx - 126.73	101.188	80.821 – 126.687	0.916
HOLE	y = 35.399logx - 16.032	73.344	41.935 – 128.279	0.976
HOLM	y = 51.022logx - 47.135	80.128	54.361 – 118.109	0.977
HOSC	y = 57.178logx - 56.081	71.659	50.678 – 101.326	0.984
HOSE	y = 48.823logx - 47.511	99.365	66.243 – 149.048	0.891
HOSM	y = 76.907logx - 100.74	91.207	70.485 – 118.022	0.991
HORC	y = 67.971logx - 87.035	103.772	77.558 – 138.847	0.966
HORE	y = 58.774logx - 58.834	71.078	50.734 - 99.580	0.970
HORM	y = 42.623logx - 40.154	130.361	81.937 – 207.404	0.970
Cyclo	y = 69.9logx - 34.936	16.409	12.006-22.305	0.995

KEY: GVLC = *Grewia villosa* leaves chloroform extract GVLE = *Grewia villosa* leaves ethyl acetate extract, GVLM = *Grewia villosa* leaves methanol extract, GVSC = *Grewia villosa* stem chloroform extract, GVSE = *Grewia villosa* stem ethyl acetate extract, GVSM = *Grewia villosa* stem methanol extract, GVRC = *Grewia villosa* roots chloroform extract, GVRE = *Grewia villosa* roots ethyl acetate extract, GVRM = *Grewia villosa* roots methanol extract, CALC = *Cassia abbreviata* leaves chloroform extract, CALE = *Cassia abbreviata* leaves ethyl acetate extract, CALM = *Cassia abbreviata* leaves methanol extract, CATC = *Cassia abbreviata* twigs chloroform extract, CATE = *Cassia abbreviata* twigs ethyl acetate extract, CATM = *Cassia abbreviata* twigs methanol extract, CASC = *Cassia abbreviata* stem chloroform extract, CASE = *Cassia abbreviata* stem ethyl acetate extract, CASM = *Cassia abbreviata* stem methanol extract, CARC = *Cassia abbreviate* root chloroform extract, CARE= *Cassia abbreviate* root ethyl acetate extract, CARM = *Cassia abbreviata* roots methanol extract, HOLC = *Hoslundia opposita* leaves chloroform extract, HOLE = *Hoslundia opposita* leaves ethyl acetate, HOLM = *Hoslundia opposita* leaves methanol extracts HOSC = *Hoslundia opposita* stem chloroform extract, HOSE = *Hoslundia opposita* stem ethyl acetate extract, HOSM = *Hoslundia opposita* stem methanol, HORC = *Hoslundia opposita* roots chloroform extract, HORE = *Hoslundia opposita* roots ethyl acetate extract, HORM = *Hoslundia opposita* roots methanol extract, Cyclo = Cyclophosphamide

Hoslundia opposita roots are used as an aphrodisiac and a remedy for colds, coughs and to relieve after-birth pains [11]. In Nigeria the leaves and whole plant have also being documented to be used in conjunctivitis, skin infection, malaria and diabetes [39]. In other parts of Africa *H. opposita* is popular for treatment of gonorrhoea, cystitis, cough, wounds, sores, snake bites, conjunctivitis, epilepsy, chest pain, stomach trouble and mental disorders [16, 17, 40]. The result from this research shows that *H. opposita* root ethyl acetate extract inhibited the growth of the tested microbes suggesting that secondary metabolites contained in this extract might be responsible for the healing power of *H. opposita* roots as used by African communities. The low cytotoxicity of some antimicrobial extracts confirms biosafety of African herbalists.

CONCLUSION

In most cases, the findings in this study validate the traditional use of *G. villosa*, *C. abbreviata* and *H. opposita* in management of microbial infections. *Hoslundia opposita* displayed cytotoxic properties which entails its candidacy in antitumor drug programmes. Further phytochemical investigations on *G. villosa*, *C. abbreviata* and *H. opposita* growing in Pare Mountains are recommended to contribute to drug development.

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