Antimicrobial activity of selected nutraceutical plants used in Northern Uganda

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Abstract:

Background: Nutraceutical plants (NP) play a vital role as supportive treatment with antiretroviral drugs (ARVs). However, there is limited scientific evidence on the efficacy of NP to justify their extensive use. This study aimed to evaluate the antibacterial activity of three nutraceutical plants which are commonly used as antimicrobials.

Methodology: Leaves of Cajanus cajan L. Millsp. and Eucalyptus globulus Labill., and stem bark of Mangifera indica L. were collected from Northern Uganda. The three samples of each NP were extracted with acetone and the minimum inhibitory concentration (MIC) values of the extracts against Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa and Klebsiella pneumoniae were determined using the serial broth microdilution technique. The mean MIC values of the extracts against each bacterial species were recorded.

Results: The three NP extracts were active against the four bacteria species with MIC ranging from 0.08 to 2.5 mg/ml. The extract of Cajanus cajan was very active against Klebsiella pneumoniae with the lowest recorded MIC of 0.08 mg/ml. The extract of Mangifera indica bark was very active against Pseudomonas aeruginosa with the lowest MIC of 0.08 mg/ml.

Conclusion: The results of the present study support the traditional use of the nutraceutical plants as antimicrobials.

Keywords: Antimicrobial, bacteria, nutraceutical plants, MIC, serial microdilution

Activité antimicrobienne de certaines plantes nutraceutiques utilisées dans le nord de l'Ouganda

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Résumé:

Contexte: Les plantes nutraceutiques (NP) jouent un rôle essentiel en tant que traitement de soutien avec des médicaments antirétroviraux (ARV). Cependant, il existe peu de preuves scientifiques sur l'efficacité des NP pour justifier leur utilisation intensive. Cette étude visait à évaluer l’activité antibactérienne de trois plantes nutraceutiques couramment utilisées comme antimicrobiens.
Méthodologie: Feuilles de Cajanus cajan L. Millsp. et Eucalyptus globulus Labill., et l'écorce de tige de Mangifera indica L. ont été collectées dans le nord de l'Ouganda. Les trois échantillons de chaque NP ont été extraits avec de l'acétone et les valeurs de concentration minimale inhibitrice (CMI) des extraits contre Staphylococcus aureus, Streptococcus pneumoniae, Pseudomonas aeruginosa et Klebsiella pneumoniae ont été déterminées à l'aide de la technique de microdilution en bouillon en série. Les valeurs moyennes de la CMI des extraits contre chaque espèce bactérienne ont été enregistrées.

Résultats: Les trois extraits de NP étaient actifs contre les quatre espèces bactériennes avec une CMI allant de 0,08 à 2,5 mg/ml. L'extrait de Cajanus cajan s'est montré très actif contre Klebsiella pneumoniae avec la valeur de CMI la plus basse enregistrée de 0,08 mg/ml. L'extrait d'écorce de Mangifera indica s'est montré très actif contre Pseudomonas aeruginosa avec la CMI la plus basse de 0,08 mg/ml.

Conclusion: Les résultats de la présente étude soutiennent l'utilisation traditionnelle des plantes nutraceutiques comme antimicrobiens.

Mots clés: Antimicrobien, bactéries, plantes nutraceutiques, CMI, microdilution en série

Introduction:

Antimicrobial resistance (AMR) to antibiotics has been reported as a major therapeutic concern (1). Based on current trends, AMR is projected to kill 10 million people worldwide each year by 2050 and will cost the global economy US $100 trillion gross domestic product (GDP) loss between 2015 and 2050 (1). AMR occurs when microbes such as bacteria, viruses, fungi and parasites transform over time and no longer respond to any drug (2) and this may be as a result of spontaneous or induced genetic mutations (1).

A weak immune system in persons with HIV infection results in susceptibility to opportunistic infections caused by opportunistic pathogens such as Candida albicans causing genital infections; Staphylococcus aureus causing eye and wound infections, pneumonia, and septicemia; Mycobacterium tuberculosis causing tuberculosis; Streptococcus pneumoniae causing pneumonia and meningitis; Klebsiella pneumoniae causing pneumonia, urinary tract infection and septicaemia (1-3).

There is no cure or effective vaccines for HIV, although people living with HIV/AIDS (PLWH) can enjoy healthy, long and productive lives by taking antiretroviral drugs (ARVs) that can effectively control the virus, manage opportunistic infections and help prevent transmission (4). PLWH exposed to subtherapeutic ARVs, are however at increased risk of developing ARV drug resistance (5). There is increased risk of AMR in PLWH across a range of bacterial pathogens and multi-drug classes (6). The use of ARVs in PLWH in Uganda is limited by toxic side effects, poor adherence to treatment, limited access and antimicrobial resistance to ARVs has been reported (7-9).

The World Health Organization (10) observed that AMR was more prevalent in cases of bacterial infections such as respiratory tract infection, diarrhoea, meningitis, syphilis, gonorrhoea and tuberculosis. PLWH had higher odds for colonization and infection with AMR S. aureus, S. pneumoniae, E. coli and K. pneumoniae (6).

One practical way to circumvent antibiotic resistance is to develop and use new antibiotics (1). There is an urgent need to discover new antimicrobial compounds or extracts to address the problem of increasing microbial resistance against current antibiotics (11). Among the potential sources of new agents are nutraceutical plants because they contain many bioactive compounds, have low toxicity and there is a long tradition of using nutraceutical plants in Uganda folk medicine (9,12). Currently drug discovery focus has moved onto plants due to beneficial attributes of plants (13). Plant based antimicrobials have immense potential to combat bacterial, fungal, protozoal and viral diseases without known side effects (13).

Nutraceutical plants play a vital role as supportive treatment with ARVs in the management of opportunistic infections associated with HIV/AIDS especially among the rural poor (9,12,13). Nutraceutical plants possess nutritional and pharmaceutical properties or a combination of both (9). Living organisms can grow, maintain themselves and reproduce by assimilation of nutritious plants that contain vitamins A, C, K, fibre, riboflavin and minerals, which are essential requirements for the health of HIV positive patients (14).

There are approximately 5,000 species of higher plants in Uganda, of which 70 are endemic (15). There are more than 200 species of non-cultivated edible plants and 75 species of edible fruit trees in Uganda, while forestland covers approximately 3.3 million hectares (15). Ethnobotanical research in Uganda has identified more than 300 trees, shrubs and herbs growing wild associated with medicinal value (15). Traditional knowledge of plants with medicinal value is passed on from one generation to another (15).

Eucalyptus globulus Labill belongs to the family of Myrtaceae, an evergreen broadleaf tall tree with a straight trunk, is cultivated in Uganda and worldwide because of adaptability and fast growth rate (16). Phytochemical analysis of leaf extract of E. globulus proved the presence of tannins, saponins, terpenoids, glycosides, alkaloids, phenolic compounds, cardiac glycosides, terpenes, reducing sugars, carbohydrates, and flavonoids (17).

Mangifera indica L. also known as Mango,
belonging to family Anacardiaceae, is a large evergreen tree growing to a height of 10-15m. The green leaves are linear-oblong and release an aromatic strong odour when crushed (16). The tree bark is thick, grey to brown in color and with age exfoliates in the form of flakes. M. indica inflorescence occurs in panicles consisting of tiny whitish-red or yellowish-green flowers. The mango fruit is a drupe (16). Selles et al., (18) reported that M. indica stem bark extracts contain ketones, phenols, terpenoids, steroids, nitrogen compounds, and one sulphur compound. The phytochemical constituents of M. indica stem bark and leaves was reported to contain alkaloids, flavonoids, saponins, tannins, phenols, and vitamins (19).

Cajanus cajan L. Millsp (also known as pigeon pea-English, Lapena-Acholi) is an erect branched hairy shrub, about 1-2m high. Green leaves are oblong-lanceolate with three leaflets, flowers are yellow in color and pod is hairy, containing 2-7 seeds (20). Cajanus cajan is an important grain-legume food crop and forage crop of rainfed agriculture in semi-arid tropics with high levels of proteins (16,20). Sahu et al., (21) reported that phytochemical analysis of the leaf, stem and seed extracts of C. cajan showed presence of saponins, tannins, alkaloids, flavonoids, anthraquinones and reducing sugars. Research found C. cajan leaves to be rich in flavonoids, saponins, tannins, reducing sugars and coumarins (20). This study aimed to provide scientific evidence on effectiveness of use of nutraceutical plants to justify their extensive use, even as widespread and empirical use of nutraceutical plants demands accurate and reliable information on efficacy (9).

Studies of in vitro screening for antibacterial activity on nutraceutical plants in Europe, Asia and Africa have been conducted on various microbial organisms causing opportunistic diseases and managed with nutraceutical plants. In Pakistan, Cassia fistula and Punica granatum have been used against fungal opportunistic infections associated with HIV (22). In Thailand, 12 medicinal plants used among HIV patients were evaluated for antibacterial activities (23). Ten Nigerian medicinal plants showed potential antymycobacterial activity following preliminary MIC assay (24). The plants A Juss Turraea floribunda and Warburgia ugandensis showed antimicrobial activity against E. coli, P. aeruginosa and Salmonella (25). Fresh extracts of Ugandan medicinal plants, Zanthoxylum chalybeum and Enceea latidens had antibiotic effects on carcinogenic and periodontopathic bacteria (26). Kuglerova et al., (27) reported antimicrobial activity of Ugandan medicinal plants.

The pharmacological activity of nutraceutical plants can be predicted by the identification of the biologically active compounds in plants called phytochemicals (28). The phytochemicals are derived from various parts of plants such as leaves, flowers, seeds, bark, roots and pulps (29,30). Nutraceutical effects of plants are attributed to the interaction of phytochemicals such as alkaloids, phenols, flavonoids, saponins and tannins (28,31). The World Health Organization (WHO) recommends that quality evaluation further involves naming the major chemical constituent, chemical structure of the selected major constituents and drawing of the chemical structures where appropriate (32). According to Chandra et al., (33), antimicrobial properties of plants are attributed to the presence of active compounds.

Different bioactive compounds with antimicrobial properties have been isolated from various nutraceutical plants. Quinones possess antimicrobial activity against Pseudomonas aeruginosa and Bacillus anthracis (33,34). Coumarins isolated from Angelica lucida, is active against the oral pathogens, Streptococcus mutans and other viridian streptococci (33). Terpenoids extracted from the bark of Acacia nilotica have antimicrobial properties and the flavonoids such as kaemferol, rutin and quercetin have antifungal properties (33). The tannin of sorghum has antimicrobial activity against S. aureus and Salmonella typhi (33). According to Aerts et al., (35), the plant Raphanus sativum has peptides which are effective antifungal against Candida albicans. Banso et al. (36) and Ragasa et al., (37) observed that terpenoids and essential oils from plants are effective against S. aureus, P. aeruginosa and viridian streptococci (34). Alkaloids from plants are effective against S. aureus, Streptococcus mutans and Microsporum canis (38). This study aimed to evaluate the antibacterial activity of three nutraceutical plants which are commonly used as antimicrobials in Uganda.

**Materials and method:**

**Study setting, sample collection and authentication:**

From the ethnobotanical study in Northern Uganda (39), three plants were selected for in vitro antimicrobial analysis based on their high frequency of mention in managing opportunistic infections associated with HIV/AIDS. Plants for analysis were collected from Pader district, located in northern Uganda at 2° 49’ 59.9” N and 33°04’ 60.0” E (15) with the total area of 3.362 square kilometres. Generally, altitude ranges between 1000-1200 metres above sea level. The district experiences tropical climate with average annual rainfall of 1507 mm and average temperatures is 23°C, vegetation is intermediate savannah grassland. Food crops grown are beans, peas, cassava, cotton, groundnuts, and sunflower. Ninety percent (90%) of the economic activity
is subsistence agriculture.

Plant specimens were identified in the field by the principal researcher (a trained Taxonomist/Botanist), based on the African Plant database and Tropical plants of East Africa catalogue (16). Plant names were checked and updated with online website (www.theplantlist.org). The voucher plant specimens were identified, collected and processed according to standard procedures (16,40). Voucher specimen were pressed, dried, mounted, coded and deposited at the Herbarium in the Department of Plant Science, Microbiology and Biotechnology, Makerere University Kampala, Uganda. Further identification of botanical specimens was done by the herbarium curator via comparison with herbarium material stored in the Makerere University Herbarium. Each plant was given an accession number; M. indica L. (MHU 41712), C. cajan L. Millsp (MHU 51151), and E. globulus Labill (MHU 51152).

Ethical approval:
Ethical approval for the study was obtained from Gulu University Research Ethics Committee (Ref. GUREC-062-20) and Uganda National Council for Science and Technology (Ref. HS983ES). Plants and seeds were collected, permission to collect was obtained from Gulu University Research Ethics Committee (Ref. GUREC-062-20) and Uganda National Council for Science and Technology (Ref. HS983ES). All local, national and international guidelines and legislation were adhered to in the conduct of this study.

Preparation of plant extract:
The three plants were evaluated for their in vitro antimicrobial activities at the Microbiology Bioscience Laboratory, Gulu University. Stem bark of M. indica L. (MHU 417 12), leaves of C. cajan L. Millsp (MHU 51151) and leaves of E. globulus Labill (MHU 51152) were air dried in shade for 10 days and later dried in oven (Memmert) at 37.2°C for 10 hours. Plant parts were ground to fine powder with a grinder (IKA Merke model M20). Plant powder was weighed on a weighing scale (OHaus Neo-tech SA). Acetone was used as an extractant in the assay because it has been shown to extract compounds of a wide range of polarities and its low toxicity to bioassay systems (41).

Approximately 10g of the plant samples were dissolved in 200 ml 99% acetone (Loba chemie PVT Ltd) in glass beaker, shaken in shaker (Stuart orbital shaker SSL1, Neotech SA) for 16 hours, and then soaked/macerated for 24 hours. The supernatants were then filtered using Whatman no. 1 filter paper. The filtrates were put in round bottom flask and evaporated to dryness using a Rotary evaporator (Hei-VAP Precision, Hei Tech), and dried extracts were transferred into pre-weighed falcon tubes and stored in freezer till further analysis.

Test microorganisms and media:
The panel of microbial organisms used were four human pathogenic species commonly causing opportunistic infections associated with HIV/AIDS and included Gram-positive cocci (Staphylococcus aureus ATCC 25923 and Streptococcus pneumoniae ATCC 49619) and Gram-negative bacilli (Pseudomonas aeruginosa ATCC 10231 and Klebsiella pneumoniae ATCC 700603) (43). The ATCC strains were purchased from Mulago Referral Hospital Microbiology Laboratory, Kampala Uganda, and handled according to performance standard procedures (42). The strains were maintained at the Gulu University Bioscience Laboratory.

Analysis was carried out in line with the guidelines and standards of Serial Microtitre Dilution Methods using tetrazolium salts, which has been shown to produce best (reproducible) results (42). All the microbial strains were sub-cultured from original culture on nutrient agar (HiMedia), kept in Eppendorf tubes in sucrose and glycerol and stored in deep freezer (Energ, Beko HS530) at -80°C. Prior to antimicrobial assay, all the strains were sub-cultured onto a fresh appropriate Muller-Hinton (MH) agar (BioLab chemicals) plate at 37°C for 24 h.

Quantitative antibacterial activity assay by minimum inhibitory concentration:
Petri dishes and microtitre culture plates were sterilised in autoclave (Sturdy SA-300 VMA). The organisms were sub-cultured onto MH agar (BioLab Chemicals) and incubated aerobically for 24 hours at 37°C in oven (Memmert). The freshly sub-cultured strains were used for the test against leaf, root and bark extracts in a biosafety cabinet. The bacterial turbidity of each species was prepared and standardized.

Inocula were prepared by transferring several single colonies of microbes to 5 ml sterile normal saline solution to produce a suspension. The turbidity of the suspension was adjusted to 1.0 McFarland standard using spectrophotometer (NeoTech SA, Jenway, Genova plus), which is equivalent to 3.0 x 10^8 CFU/ml.

Preparation of crude extracts and antibiotics, and determination of MIC:
The dried plant extract was reconstituted in 99% acetone (Loba chemie PVT Ltd) to make 10 mg/ml stock extract (42). Stock extract was serially diluted 1:1 with sterile distilled water. 1 ml of the final extract concentrations of 5, 2.5, 1.25, 0.63, 0.32, 0.16, 0.08, 0.04 mg/ml was made in culture plates.
100µl concentration of the plant extract were added to the wells of the sterile 96-well microtitre plate. A positive control of a standard antibiotic ciprofloxacin (Rx Farma, Hertfordshire, UK) was also serial diluted accordingly. Acetone and water were used as negative control. After the dilution process, 100µl of Mueller-Hinton broth (Accomix chemicals) for bacteria was added to each well, followed by 100µl of test organisms. The microtitre plates were covered and incubated at 37°C for 24 hours.

The minimum inhibitory concentration of the extract was determined following addition of 40µl of 0.2mg/ml 2,3,5-triphenyltetrazolium chloride (TTC) (Loba Chemie) for colorimetric assay, incubated at 37°C and observed for 4 hours. Microbial growths were determined by observing the change of color of TTC in the microplate wells, with pinkish-red formazan when there is growth, and clear solution when there is no growth. The colourless well immediately after a red well was recorded as the MIC, which is the lowest extract concentration showing no color change (clear) indicating complete inhibition of bacterial growth (42). The experiments were carried out in triplicate, and the mean MICs were recorded.

**Statistical analysis:**
The results were expressed as mean MICs of 3 replicates. Descriptive statistics and the ANOVA single factor statistical tool was used to analyze results of the MICs of plant extracts from the serial microdilutions. Statistical significance was defined at p<0.01 level (42).

**Results:**
All the nutraceutical plants exhibited low MICs against the bacteria strains. The serial microdilution results by single factor analysis of variance (ANOVA) indicated that there is significant difference in the sensitivity of the tested microorganisms to the various extracts (p<0.01). The microbial sensitivity to different extracts represented by mean MICs values ranged from 0.08 to 2.5 mg/ml (Table 1 and Fig 1). *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (MIC=0.08mg/ml) were the most sensitive, followed by *Streptococcus pneumoniae* (MIC=0.16mg/ml).

**Antibacterial activity of Mangifera indica stem bark extract:**
The bark extract of *M. indica* exhibited strong antimicrobial activity against *S. aureus* and *P. aeruginosa* in this study. Antibacterial tests results indicated that *M. indica* bark extract inhibited growth of *S. aureus* and *P. aeruginosa* at mean MICs of 0.63 mg/ml and 0.08 mg/ml respectively.

**Antibacterial activity of Cajanus cajan leaf extract:**
The leaf extracts of *C. cajan* exhibited growth-inhibitory activity against *S. pneumoniae* and *K. pneumoniae* that causes pneumonia. *Cajanus cajan* leaf extract inhibited the growth of *S. pneumoniae* and *K. pneumoniae* at MICs of 0.16 mg/ml and 0.08 mg/ml respectively.

**Antibacterial activity of Eucalyptus globulus leaf extract:**
*Eucalyptus globulus* exhibited growth-inhibitory activity against *S. pneumoniae* and *K. pneumoniae* at MICs of 1.25 mg/ml and 2.5 mg/ml respectively.

**Discussion:**
Our results of the antibacterial activity

<table>
<thead>
<tr>
<th>Plant species</th>
<th>Bacteria strain</th>
<th>Mean MIC (mg/ml)</th>
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<tbody>
<tr>
<td>Mangifera indica bark extract</td>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em> ATCC 10231</td>
<td>0.08</td>
</tr>
<tr>
<td>Cajanus cajan leaf extract</td>
<td><em>Streptococcus pneumoniae</em> ATCC 49619</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em> ATCC 700603</td>
<td>0.08</td>
</tr>
<tr>
<td>Eucalyptus globulus leaf extract</td>
<td><em>Streptococcus pneumoniae</em> ATCC 49619</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em> ATCC 700603</td>
<td>2.5</td>
</tr>
<tr>
<td>Ciprofloxacin (standard)</td>
<td><em>Staphylococcus aureus</em> ATCC 25923</td>
<td>0.04</td>
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<tr>
<td></td>
<td><em>Pseudomonas aeruginosa</em> ATCC 10231</td>
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<td><em>Streptococcus pneumoniae</em> ATCC 49619</td>
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<tr>
<td></td>
<td><em>Klebsiella pneumoniae</em> ATCC 700603</td>
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of *M. indica* stem bark extract agrees with the findings of Bbosa et al., (44), who reported antimicrobial effect of ethanolic extract of *M. indica* against *S. aureus, E. coli* and *P. aeruginosa* with MIC range of 5.48 to 43.75 mg/ml. Mushore and Matuvhunye (45) also reported that stem bark extract of *M. indica* had antimicrobial activity against *S. aureus* using broth dilution MIC method of 0.16-1.25 mg/ml. *Mangifera indica* stem bark extract inhibits the growth of microorganisms with varying degrees of susceptibility depending on the bacterium and extract, with MIC values ranging from 6.25-50 mg/ml (46).

The antimicrobial activity in our study supports the claim by local communities for use of *M. indica* stem bark decoctions for treating opportunistic infections such as diarrhoea and gastrointestinal infections. Aqueous and methanolic extracts of *M. indica* stem bark possess antimicrobial and anti-diarrhoeic properties with methanolic extract MIC being 256 µg/ml for both Gram-positive and Gram-negative bacteria (43). The study of Osei-Djarbeng et al., (47) showed that bark and leaf extracts of *M. indica* has antimicrobial activity. *Pseudomonas aeruginosa* and *S. aureus* showed susceptibility to inhibitory activity of *M. indica* bark extract (48). Sanusi et al., (46) reported that the antimicrobial activity obtained in their study indicated presence of bioactive compounds and support the claim by the local communities for the use of *M. indica* stem bark decoction for treatment of infections such as diarrhoea. *Mangifera indica* bark extract showed significant activity against four clinical strains of *S. typhi, B. subtilis, E. coli* and *K. pneumoniae*, where all solvent extracts at dose range 2-4 mg/ml showed significant antibacterial activity (49). Phytochemical screening of crude stem bark extracts of *M. indica* revealed the presence of tannins, saponins, alkaloids, flavonoids, cardiac glycosides and phytosterols (46). This could be said to be responsible for the efficacy of *M. indica* bark studied in the treatment of different diseases.

Our findings on antibacterial activity of *C. cajan* leaf extract are in agreement with previously published research by Pratima and Mathad (50), who reported that extract of *C. cajan* inhibited growth of both Gram-positive and Gram-negative bacteria (*S. aureus, S. pneumoniae, K. pneumoniae*). *Cajanus cajan* showed antibacterial activities against *Streptococcus mutans* (51). The extracts of *C. cajan* showed potential activity against *S. pneumoniae, S. aureus*, and *P. aeruginosa* (52) and is capable of preventing and treating bronchitis, cough, pneumonia and respiratory infections (53). Oke (54) reported *C. cajan* leaves to contain alkaloids, flavonoids, tannins, saponins, terpenes, phlobatannins, anthraquinones and sterols. Mohanty et al., (55) found steroids, phenolic compounds, saponins, glycosides, flavonoids in *C. cajan* and this could be said to be responsible for the efficacy of *C. cajan* leaves studied in the treatment of different diseases.

The results obtained on antibacterial activity of *E. globulus* leaf extract are in agreement with those obtained by Mulyaningsih et al., (56), who reported that *Eucalyptus globulus* exerted promising antibacterial activity against methicillin resistant *S. aureus* (MIC 250 µg/ml), and Cermelli et al., (57) who observed that *S. pneumoniae* was susceptible
to antibacterial activity of *E. globulus*. Bachir and Benali (58) reported that essential oil in leaves of *E. globulus* has antimicrobial activity against Gram-negative and Gram-positive bacteria. Alvarenga et al., (59) also reported that 32 air-bone anti-tuberculosis components were identified in *Eucalyptus citriodora*. Eucalyptus essential oil was effective against *Staphylococcus* and *Streptococcus* (60). Phytochemical analysis of leaf extract of *E. globulus* proved the presence of tannins, saponins, terpenoids, glycosides, alkaloids, phenolic compounds, cardiac glycosides, terpenes, reducing sugars, carbohydrates, flavonoids (18) and this could be said to be responsible for the efficacy of *E. globulus* studied in the treatment of different diseases.

**Conclusion:**

The results of the present study support the traditional use of the studied nutraceutical plants for the management of opportunistic infections associated with HIV/AIDS. We confirmed the ability of selected plant extracts to inhibit bacterial growth. The antimicrobial activity obtained in this study indicated presence of bioactive compounds and support the claim by the local communities for the use of plant parts decoction for treatment of opportunistic infections such as diarrhoea, tuberculosis, oral candidiasis, pneumonia. Our results provide useful baseline information for the potential use of the studied nutraceutical plants in the fight against ARV drug resistant bacteria and the possibility of developing plant-based drugs to help in long term management of opportunistic infections associated with HIV/AIDS.

A high degree of medical pluralism has been observed among local community, therefore communication and collaboration between biomedical clinicians and traditional medicine practitioners should be encouraged. We recommend that the Ministry of Health in Uganda should coordinate this collaboration. Bakibinga (61) reported that contemporary Intellectual Property Law permits only the patenting of an identified “active principle” from a plant, and not the plant or folk information relating to medicinal properties of a plant, according to Copyright and Intellectual Property Rights (CIPR). Therefore, we recommend that the issues of traditional medicine practitioners should be fully exploited with the help of Gulu University and the Registrar of Patents office in Uganda.

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**Contributions of authors:**

NI was involved in study conceptualization, funding acquisition, methodology, investigation, data curation, validation, formal analysis, writing original draft and editing, and visualization; AL, ATM and EN were involved in reviewing and editing of manuscript and supervision of the project. All authors read and approved the final manuscript.

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**Conflict of interests:**

Authors declare no conflict of interest.

**Availability of data and materials:**

The datasets during and/or analysed during the current study are available from the corresponding author on reasonable request.

**References:**

1. Robbers, J. E., Speedie, M. K., and Tyler, V. E. Pharmacognosy and Pharmacobiotechnology. Williams & Wilkins, 1996
8. Mustapha, A. A. Ethnobotanical field survey of


35. https://doi.org/10.4314/njhbs.v8i2.60933


37. https://doi.org/10.3390/ijms13020016


Antimicrobial activity of nutraceutical plants


