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Antioxidant and Antibacterial Activity of Methanol Extract of *Momordica Balsamina*

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ABSTRACT

Momordica balsamina is considered as a miracle herb used in African traditional medicine due to its tremendous medicinal and nutritional properties. In this present study we evaluated the antioxidant and antimicrobial activities of the methanol extract of *Momordica balsamina* (MEMB). Phytochemical screening followed by thin layer chromatography and spectrophotometrydone to measure the radical scavenging activity of 2,2-diphenyl-l-picrylhydrazyl (DPPH). Total phenolic content and total antioxidant activity of MEMB was determined. Zones of inhibition, minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC) were measured by agar diffusion and liquid broth dilution assays to assess the antimicrobial activities of MEMB. Phytochemicals isolated were flavonoids, tannins, coumarins, terpenoids and phenols. The antioxidant potential and antimicrobial activity of MEMB can be attributed to the total phenolic content and other bioactive phytochemicals.MEMB showed antimicrobicidal activity more against Gram positive than Gram negative organisms. *S.agalactiae,S.aureus* (ATCC 25923) and *L.monocytogenes* were more susceptible than *Proteus mirabilis*, *E.coli, K.pneumoniae, P.aeruginosa or S. typhimurium.* MEMB also showed antifungal activity against *C.albicans* species though at a higher concentration[MIC - 0.938mg/ml and MBC-1.875mg/ml]. MEMB can therefore be considered as a potential medication in the management of infectious diseases.

Key words: *Methanol extract of Momordica balsamina, phytochemicals*, *antioxidants, total phenolic content, antimicrobial activity, Botswana.*

1.0 Introduction

Momordica balsamina (aka Balsam apple, bitter cucumber or bitter melon, African pumpkin)is a miracle herb which has tremendous medicinal and nutritional properties(Kaur *et al.*, 2012).It is a high-climbing vine from family Cucurbitaceae, fairly common and widespread in Namibia, Botswana, Swaziland and all the provinces of South Africa except the Western Cape. It is also indigenous to tropical Africa, Asia, Arabia, India and Australia(Singh Thakur *et al.*, 2009; Ramalhete *et al.*, 2011; Thakur *et al.*, 2011).

The leaves of *Momordica balsamina* are an important source of nutrients including 17 amino acids, various minerals like potassium, magnesium, phosphorus, calcium, sodium, zinc, manganese, iron and vitamins A and C. Its high potassium content is a good source for the management of hypertension and other cardiovascular conditions (Karumi.Y, Onyeyili PA, 2004; Flyman and Afolayan, 2007; Singh Thakur *et al.*, 2009).

Momordica plants also contain biologically active phytochemicals, such as resins, alkaloids, flavonoids, anthraquinone, steroids, glycosides, terpenes, saponins and carbohydrate(Singh Thakur *et al.*, 2009). Flavonoids and phenolic compounds are considered as antioxidants due to their free radical scavenging activity and lipid peroxidation inhibition activity. Phenolic compounds are involved in conferring plants with oxidative stress tolerance and flavonoids suppress reactive oxygen formation, chelate trace elements involved in free-radical production, scavenge reactive species, and up-regulate and protect antioxidant defense(Baba and Malik, 2014).Antioxidants are capable of stabilizing, or deactivating free radicals before the latter attack cells and biological targets, The antioxidant potential of a plant is assessed by determining the total phenolic content and generation of the ABTS radical action(Akula and Odhav, 2008). *M. balsamina* has also been reported to be a potent inhibitor of HIV-1 replication *in vitro.* due to the presence of ribosome inactivating proteins, Momordin I and II, by significantly increasing the CD4 count within two weeks (Singh Thakur *et al.*, 2009).

Various studies have reported that the extract of the *M. balsamina* plant has antiviral, anti-inflammatory, anti-diarrheal, antiseptic, antimicrobial, antidiabetic and anti-plasmodial activity and is being used in African traditional medicine(Benoit-Vical *et al.*, 2006; Ramalhete *et al.*, 2011; Singh Thakur *et al.*, 2009).

This present study evaluated the phytochemicals, antioxidant potential, total phenolic content, and antimicrobial activities of the methanol extract of *Momordica balsamina (*MEMB).

2.0 Materials and Methods

2.1 Collection and identification of plants

Identification of the aerial parts of *Momordica Balsamina,* collected locally from Botswana was done at the University of Botswana Herbarium by Dr. M. P. Setshogo. The voucher number for the submitted specimen was (G2016/, A02).

2.2 Preparation of the methanol extract

Methanol extract of *M.balsamina* was obtained from the plant parts as detailed elsewhere using 70% methanol and Buchi type rotary vacuum evaporator (Souda *et al.*, 2016).

2.3 Chemicals

All the chemicals, solvents and reagents like DPPH (2,2-diphenyl-l-picrylhydrazyl), Folin-Ciocalteau reagent, gallic acid (AR),ascorbic acid and anhydrous sodium carbonate used were analytical grade and bought from Sigma-Aldrich Chemical Company, (St. Louis, MO) USA., Fluka Chemicals (Steinheim, Germany), Rochelle Chemicals (South Africa). Unilab (South Africa). The silica gel coated aluminium backed TLC sheets were ready made (Souda *et al.*, 2016; Mannathoko *et al.*, 2017).

2.4 Phytochemical screening

The screening tests were performed in duplicates as detailed by Mazimba (2015), for flavonoids, tannins, saponins, coumarins terpenoids, fatty acids, phenols, aminoacids, alpha proteins, quinones, oxalates(Mazimba *et al.*, 2015; Souda *et al.*, 2016).

2.5. Antioxidant Status

The methods used to determine the free radical scavenging effects and in turn the antioxidant property were the TLC - Semi Quantitative DPPH Assay described by Juma and Majinda, (2004), the spectrophotometric method using DPPH, modified by Yeboah and Majinda, (2009)**,** the total phenolic content by method described by Yeboah and Majinda (2009) and ABTS radical scavenging activity described in detail by Pellegrini*et al.*,(1999)(Saramma and Padmaja, 2013; Souda *et al.*, 2016; Mannathoko *et al.*, 2017).

2.6. Antimicrobial activity

2.6.1. Microbial cultures

The antimicrobial activity of MEMB was tested using ATCC strains and a clinical isolate obtained from the Department of Microbiology, School of Allied Health Professions, University of Botswana, Botswana. The microorganisms included **Gram positive cocci** : *Staphylococcusaureus(ATCC 25923), Methicillin resistant Staphylococcus aureus (ATCC 43300), Staphylococcus aureus* from a patient sample, *Staphylococcus epidermidis(ATCC12228) and Streptococcus agalactiae (ATCC 27956),* **Gram positive bacilli:** *Listeria monocytogenes,* **Gram negative bacilli:** *Escherichia coli (ATCC10536), Klebsiella pneumoniae(ATCC700603), Proteus mirabilis(ATCC 25933), Pseudomonas aeruginosa(ATCC 27853), Salmonella typhimurium(ATCC 14028) and a* **fungus***: Candida albicans(ATCC 90028).* All organisms were tested for purity and maintained in nutrient agar plates (OXOID).

2.6.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing to the different microorganisms were carried out by well diffusion on Mueller Hinton agar plates (MHA) (MAST) with some modification(Marie B. Coyle, 2005)and also reported elsewhere(Souda *et al.*, 2016; Mannathoko *et al.*, 2017).In brief, 6mm diameter wells were made in the MHA agar plate with the base of a sterilized glass Pasteur pipette. Lawn cultures of each of the bacterial suspensions in Tryptone Soya broth (OXOID), adjusted to 0.5 McFarland standard turbidity, (equivalent to a bacterial suspension of 1.5 X 10^8 colony forming units per ml (CFU/ml)), were made on the MHA plates. 100 microliters (µl) of MEMB, dissolved in 10% dimethyl sulfoxide (DMSO), which gives a concentration of 20mg, was added to each well. DMSO (100µl) was also added to the wells in each plate as the control. The tests were done in duplicates.

Ampicillin (10µg) (Mast Diagnostics) discs were used as the positive control for each of the organism, meropenem (10µg) for *Staphylococcus aureus*(ATCC 43300), ceftazidime (30µg) for *Pseudomonas aeruginosa,*co-trimoxazole (CTX 25µg) for *Klebsiella pneumonia* and fluconazole (25µg) for C.*albicans*. 20µl of DMSO was used as the negative control.

The plates were incubated at 37°C in an incubator for 24 hours and zones of inhibition measured using Vernier calipers.

2.6.3. Minimum Inhibitory Concentration
Micro well dilution method with some modifications (Marie B. Coyle, 2005) was done to determine the minimal inhibitory concentration (MIC) of the extract for each of the microorganisms using 96 well plates (Souda *et al.*, 2016; Mannathoko *et al.*, 2017).

The lowest concentration of the sample that prevented visible growth after 24 hours incubation at 37°C, indicated by turbidity, was considered as the MIC of the extract.

The minimum bactericidal concentrations (MBC) were also determined by subculturing the suspension from all the wells on to a MHA plate and incubated at 37° C in ambient air for 24 hours. The MBC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms(Nakamura *et al.*, 1999). The tests were done in duplicate.

The dilution assays are more reliable than diffusion assays due to many factors (Rios, Recio and Villar, 1988) The MBC values are more reliable than the MIC values which depends only on the visual observation of turbidity (Junaid et al.2006). MIC values <1mg/ mL expressed by crude plant extracts are regarded as indicators of good antimicrobial activity with potential physiological relevance *in vivo*(Mann, 2012)

2.7. Statistical analysis
All data were expressed as the mean ±S.E and standard deviation.
3. Results
3.1. Phytochemical screening of MEMB.

Table 1: Phytochemical screening of MEMB

Test	MEMB
Flavonoids	+
Tannins	+
Saponins	-
Coumarins	+
Terpenoids	+
Fatty acids	-
Phenols	+
Amino acids	-
α proteins	-
Quinones	-
Oxalate	-

3.2. Antioxidant Assay status
3.2.1. TLC - Semi Quantitative DPPH
In the presence of an antioxidant molecule, the free radical, DPPH in the MEMB is reduced, giving rise to a colorless methanol solution. The decrease in the concentration of DPPH radical due to antioxidant activity of the MEMB and the standards used, gallic acid and ascorbic acid is shown in Fig 1.

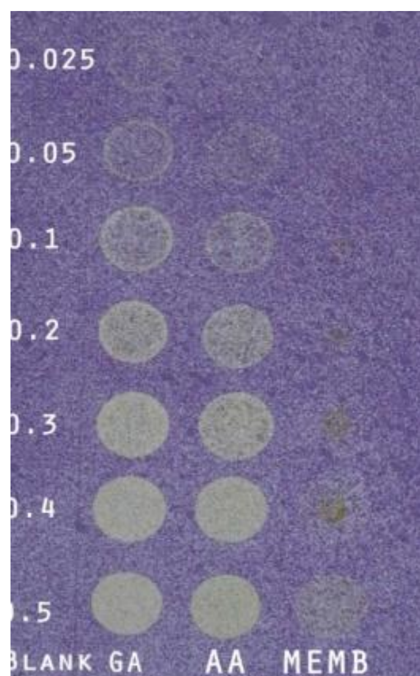

Fig 1: **TLC- Semi quantitative DPPH activity of MEMB.**

3.2.2. DPPH Spectrophotometric method

The free radical scavenging activity of *M.balsamina* is determined from the reduction in absorbance at 517 nm and compared with the standard gallic acid and ascorbic acid, as shown in Fig 2 using the formula DPPH Inhibition %= Absorbance control- Absorbance sample) /Absorbance control x 100.

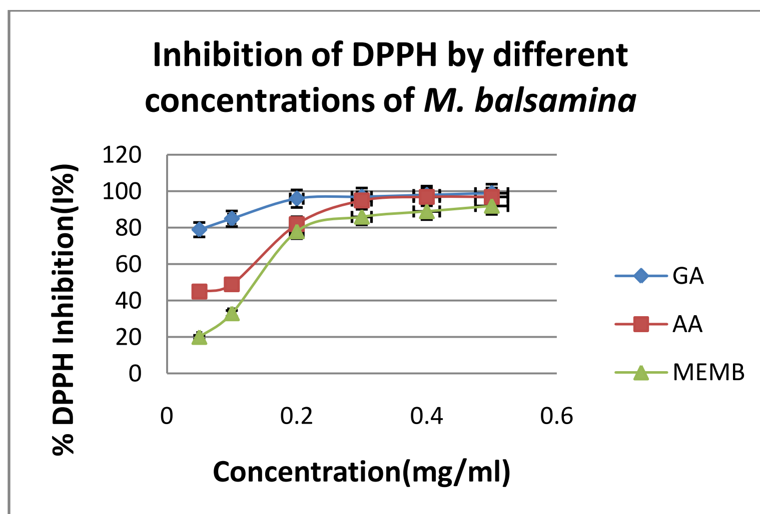

FIG 2: Radical scavenging activity of MEMB

(GA- Gallic acid, AA -Ascorbic acid, methanol extract of *M.balsamina*- MEMB)

3.2.3 Total phenolic content:

TPC of the MEMB was calculated from the linear regression equation of the standard curve y =36.84 x+0.1069 using the equation (X = (Y - 0.064) / 0.094 X 100)(Souda *et al.*, 2016).From this equation the equivalent concentration of gallic acid 254.21± 0.43 mg/ mL was determined for each extract and converted to mg of gallic acid equivalents/g of dry extract (mg GAE/g).

Table 2: Total Phenolic content

Concentration (mg/ml)	TPC (mg GAE/100g) Momordica
0.1	9.84
0.2	17.02
0.3	26.24
0.4	38.3
0.5	49.29

3.2.4. ABTS radical scavenging activity

The method is a decolorisation assay carried out by adding DMSO diluted solution containing ABTS and potassium persulphate to different concentration of the MEMB and absorbance read at 734 nm along with the reference standard gallic acid and ascorbic acid and percentage inhibition calculated.

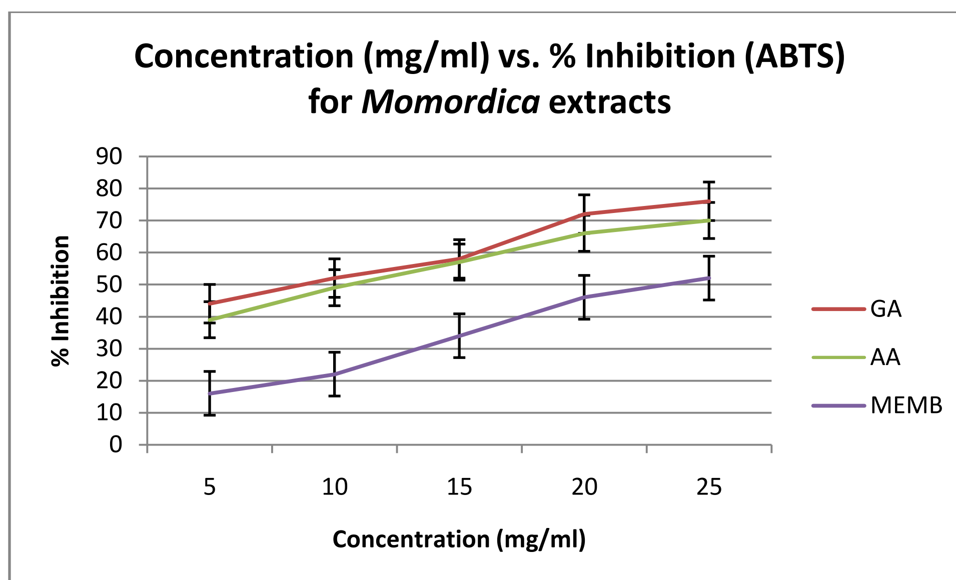

Fig 3: ABTS radical scavenging activity

(GA- Gallic acid, AA -Ascorbic acid, MEMB- Methanol extract of *Momordica balsamina*)

3.3 Antimicrobial activity

The activity of MEMB against the different microorganisms was examined and their antimicrobial activity was determined by the presence or absence of inhibition zones around the well, zone diameter (Table 3), MIC values and MBC values (Table 4).

MEMB showed variable antimicrobial activity against all the microorganisms tested using well diffusion method. Considerable anti-microbial activity was noted against the Gram positive cocci *Streptococcus agalactiae(18mm)* and Gram negative bacilli *Proteus mirabilis (20mm)*, although not comparable to the controls. The diameters of the zone of inhibition for all the other microorganisms were ≤15mm.

Table 3: Antimicrobial activity of MEMB by the well-diffusion method.

Organisms	Well diffusion (100µl=20mg/ml Zone of inhibition (mm)	Control (Ampicillin 10µg) Zoneof inhibition(mm)
Gram positive Organisms		
Staphylococcus aureus (ATCC 25923)	14	33
Staphylococcus aureus (Methicillin resistant) (ATCC 43300)	11	29[a]
Staphylococcus aureus. (patient sample)	15	26
Staphylococcus epidermidis (ATCC 12228)	15	28
Streptococcus agalactiae (ATCC 27956)	18	34
Listeria monocytogenes	12	37
Gram negative Organisms		
Escherichia coli (ATCC 10536)	12.5±0.5	19
Klebsiella pneumoniae (ATCC 700603)	10	23[b]
Proteus mirabilis (ATCC 12228)	20	30
Pseudomonas aeruginosa (ATCC 27853)	13	27[c]
Salmonella typhimurium (ATCC 14028)	13.5±0.5	24
Fungus		
Candida albicans (ATCC 90028)	15 ± 1	25[d]

[a]Meropenam (10ug), [b]CTX (25ug) [c]Ceftazidime (30ug), [d] Fluconazole (25ug).

Table 4: Antimicrobial activity of MEMB by micro dilution assay, (MIC and MBC).

Organisms	MIC (mg/ml)	MBC (mg/ml)
Gram positive Organisms		
Staphylococcus aureus (ATCC 25923)	0.234	0.938
Staphylococcus aureus (Methicillin resistant) (ATCC 43300)	0.469	1.875
Staphylococcus aureus. (patient sample)	0.938	1.875
Staphylococcus epidermidis (ATCC 12228)	0.938	1.875
Streptococcus agalactiae (ATCC 27956)	0.234	0.234

Listeria monocytogenes	0.234	0.469
Gram negative Organisms		
Escherichia coli (ATCC 10536)	1.875	1.875
Klebsiella pneumoniae (ATCC 700603)	0.469	1.875
Proteus mirabilis (ATCC 12228)	0.234	0.938
Pseudomonas aeruginosa (ATCC 27853)	0.234	1.875
Salmonella typhimurium (ATCC 14028)	0.469	0.938
Fungus		
Candida albicans (ATCC 90028)	0.938	1.875

The range of MIC for Gram positive and Gram negative organisms was between 0.234 – 0.938mg/mL and 0.234 – 1.875 mg/mL respectively. The MEMB had an MIC of <1mg/mL against all organisms tested in this study, except *E.coli.*

The range of MBC for the Gram positive bacteria was from 0.234 mg/mL – 1.875 mg/ml and for Gram negative bacteria from 0.938 mg/mL- 1.875 mg/mL. The Gram positive bacteria *Streptococcus agalactiae, Listeria monocytogenes*, *Staphylococcus aureus* (ATCC 25923) and the Gram negative organisms, *Proteus mirabilis* has an MBC of < 1mg/mL indicating good antimicrobial activity of MEMB.

Antifungal activity of MEMB was shown by its activity against *Candida albicans*, with MIC of 0.938mg/ml, MBC of1.875 mg/ml and zone of inhibition of 15 mm.

The solvents used for the dilutions were also tested for antimicrobial activity and was found to have no inhibitory activity.

4. Discussion

Phytochemicals:
In our study, the phytochemicals isolated were flavonoids, tannins, coumarins, terpenoids and phenols similar to other reported studies(Singh Thakur *et al.*, 2009). Saponin was absent in our extract(Karumi.Y, Onyeyili PA, 2004).
The uncontrolled production of oxygen free radicals and the unbalanced mechanism of antioxidant protection result in the onset of many diseases. The antioxidants when present at low concentrations compared to that of an oxidizable substrate, significantly delay or inhibit the oxidation of that substrate(Akula and Odhav, 2008). Free radical and hydroxyl free radical-scavengers prevent damage to cellular components arising as a consequence of chemical reactions and are critical for maintaining optimal cellular and systemic health and wellbeing (Singh Thakur *et al.*, 2009).Most antioxidants isolated from higher plants are phenolic compounds having carbon-based aromatic phenyl-ring compounds which are easily oxidized to quinones by reactive oxygen species, a property that helps to account for their free radical scavenging capacity. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. Phenolic compounds also have a metal chelation property and lipoxygenase inhibitory properties that have been used for the treatment of inflammatory diseases. (Akula and Odhav, 2008).

In the radical scavenging studies using DPPH, MEMB almost completely inhibited DPPH absorption (92%).Similar result was reported along with significant inhibition of 5-lipoxygenase (5-Lox) activity by Singh Thakur, (2009).

Antimicrobial activity:

The methanol extract of *M.balsamina*, in this study also, showed antimicrobicidal activity more against Gram positive than Gram negative organisms, as indicated by the MIC and MBC which is similar to reports from other studies(Singh Thakur *et al.*, 2009; Ajji, Walder and Puri, 2016)

The phytochemicals present in the plant extracts like phenols, flavonoids and tannins have been reported to complex with proteins and polysaccharides leading to inactivation of microbial adhesion enzymes, cell envelope, and the transport protein accounting for its antimicrobial activity(Singh Thakur *et al.*, 2009).

Gram positive organisms have been reported to be more susceptible to phenols which are the predominant active chemical compounds in the plant extracts. The phenolic component can cause leakage of intracellular ATP and potassium ions leading to bacterial cell death. The difference in the susceptibility of Gram positive and Gram negative bacteria to the extract of *M.balsamina* is probably because of the difference in the outer membrane structure of the Gram positive and Gram negative bacterial cell wall as reported also by other studies(Obinna *et al.*, 2009; Joshi, 2013).Gram-positive bacteria contain an outer peptidoglycan layer, which is an ineffective permeability barrier(Baba and Malik, 2014).Gram negative bacteria have a hydrophilic outer membrane which blocks penetration of hydrophobic compounds into the cell membranes(Mannathoko *et al.*, 2017). The flavonoids complexes with bacterial cell wall and disrupt the membrane integrity. It has also been shown that flavonoids interfere with various bacterialvirulence factors, including enzymes, toxins and signal receptors(Cushnie and Lamb, 2005). Tannins have been reported to inhibit microbial adhesion enzymes and cell envelope proteins.Tannins also have been shown to have antiviral activity by inhibiting viral reverse transcriptase (Mann, 2012).

Another active component found in the MEMB, is a ribosome inhibiting protein (RIPs) called balsamin, a class of defense proteins reported to have a DNase like, antibacterial activity against *S. aureus, S. epidermidis, S.enterica, E. faecalis, E. coli* and *P. aeruginosa*(Kaur *et al.*, 2012; Ajji, Walder and Puri, 2016).

Cucurbitane-type triterpenoids compounds, isolated from *M. balsamina* also have been shown to inhibit the efflux pump system of Gram-positive bacteria. They are ineffective against the efflux pump systems of Gram negative bacterial strains like *S.typhimurium* and *E. coli* strains This may be due to the difference in the outer membrane and its lipopolysaccharide layer that can act as an effective barrier to these compounds or to RND-type efflux pumps that are not affected by the compounds studied (Ramalhete *et al.*, 2011).

In this study, we found that among the Gram positive bacteria studied, *S.agalactiae* was more susceptible to the antimicrobial effect of the extract. An almost equal susceptibility was noticed *for S.aureus*(ATCC 25923) and*L.monocytogenes*. Among Gram negative organisms, *P. mirabilis* was more susceptible than *E.coli, K.pneumoniae, P.aeruginosa orS. typhimurium.*

The methanol extract of M.balsamina also showed antifungal activity against *C.albicans* species though at a higher concentration [MIC - 0.938mg/ml and MBC-1.875mg/ml]. Momordica plants produce a number of proteins and peptides that are indicative of antifungal activity, including trypsin inhibitors, lectins, ribosome-inactivating proteins and ribonucleases (Burger *et al.*, 2010).

5. Conclusions

The phytochemicals and proteins like RIP's, balsamin, peptide and lectins found in the extracts of M.balsamina plant are shown to have antioxidant, antimicrobial, antifungal and antiviral properties. These substances contribute to its medicinal value and rationalize the use of the plant extract as traditional medicines in the treatment of infections due to bacteria, fungus, and viruses. They can also be valuable in the management of non- communicable diseases like diabetes, hypertension and cancer. Studies on the preparation, effective doses and side effects of these extracts are warranted.

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Consent: Not applicable. **Ethical Approval:** Not applicable.

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