Original Article

Evaluation of Nutraceutical Bottle Gourd (*Lagenaria siceraria*) as a Potential Source of Natural Antimicrobial Agent

Disha Menpara, Dishant Desai, Tejas Rathod and Sumitra Chanda*

Phytochemical, Pharmacological and Microbiological Laboratory Department of Biosciences, Saurashtra University - Rajkot, 360 005, Gujarat, India

Address for Correspondence

Phytochemical, Pharmacological and Microbiological Laboratory Department of Biosciences, Saurashtra University-Rajkot, 360 005, Gujarat, India.

E-mail: svchanda@gmail.com

ABSTRACT

The antimicrobial activity of different solvent extracts (hydro alcoholic extracts of methanol) of different parts of *Lagenaria siceraria* (Molina) Standl was evaluated for its antimicrobial potential. The *in vitro* antimicrobial activity was evaluated by agar well diffusion method. Extraction was done by individual cold percolation method. The antimicrobial activity was tested against five Gram positive bacteria, five Gram negative bacteria and four fungi. The MIC and MBC was also determined, including five antibiotics. All the parts possessed antimicrobial activity but to a different level. The extracts showed better antifungal activity than antibacterial activity and Gram negative bacteria were more susceptible than Gram positive bacteria. The peel showed lowest MIC and MBC values indicating the therapeutic value of agro waste material.

Keywords: *Lagenaria siceraria*, hydro alcoholic extracts, agro waste, antimicrobial activity.

INTRODUCTION

Medicinal plants have been age long remedies for human diseases because they contain components of therapeutic value. Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is most popular for 80% of the world population even today. Plants synthesize chemicals as parts of their defense against many diseases including malaria, epilepsy, infantile convulsion, diarrhea, and dysentery, fungal and bacterial infections[1]. Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in pharmaceutics. Herbal drugs have gained importance in recent years because of their efficacy and cost effectiveness.

Nowadays multiple drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. In addition to this,

problems are sometimes associated with adverse effects on the host including hypersensitivity, immune-suppression and allergic reaction; this situation forced scientists to search for new antimicrobial substances. There is a constant need for new and effective therapeutic agents. Antimicrobials with plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of side effects that are often associated with synthetic antimicrobials. Synthetic antimicrobial drugs are widely used, but they are sometimes causing adverse drug effects and are also responsible for some toxic effects[2]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. Herbal medicines are considered as time tested and are relatively safe for both human use and environment friendly[3]. They are also cheap, easily available and affordable. There has been considerable interest in the use of plant extracts as multi-drug complex as an alternative method to control pathogenic microorganisms[4,5]. There are many reports that different plant parts such as bark, leaves, peel, seed, and stem may potentially possess antimicrobial property[6-9].

Nowadays, food industries need new food ingredients obtained from natural sources, to develop novel functional foods or nutraceuticals. Fruits and vegetables apart from being good sources of vitamins, minerals, fiber are also rich source of secondary metabolites. Generally, fruits and vegetable peels are thrown away into the environment which only leads to environment pollution. If such waste materials is reused by converting them into some value added applications like biofuels, source of antioxidants/antimicrobics, it will be very fruitful.

Considering the above, different parts of *Lagenaria siceraria* (Molina) Standl a common vegetable consumed in India was selected to evaluate its antimicrobial potency. *Lagenaria siceraria* (Molina) Standl belongs to the family Cucurbitaceae. The fruits are widely used in Ayurveda and other folk medicines traditionally used for its cardioprotective, cardiotonic, general tonic, diuretic, aphrodisiac, antidote to certain poisons, scorpion strings, alternative purgative, scorpion strings, and cooling effects. It cures pain, ulcers and fever and used for pectoral cough, asthma and other bronchial disorders especially syrup prepared from the tender fruits[10]. Some of the reported activities are antihepatoxic activity[11], hypolipidemic and anti-hyperlipidemic effects[12], diuretic activity[13], cardioprotective activity[14] and antioxidant activity[15].

MATERIAL AND METHODS

Collection of the plant material

The fruits and aerial part (Leaf and stem) of *L. siceraria* (Fig. 1) (SU/BIO/516/Thakrar) was collected in September, 2011 from Rajkot, Gujarat, India and identified by comparison with specimens available at the Herbarium of the Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The aerial parts and fruits were separated, washed thoroughly with tap water. The peels of the fruits were gently separated and peel, pulp and aerial parts were shade dried, homogenized to fine powder and stored in air tight bottles.

Extraction

Cold percolation extraction method

The dried powder of different parts was extracted individually by cold percolation method[16]. The solvents used were 100% methanol, 75% methanol 50%

methanol, 25% methanol and water (aqueous). Ten grams of dried powder was taken in 100 ml of hexane in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min. Supernatant was collected and the solvent was evaporated. The residue was then added to 100 ml of solvent (100% methanol, 75% methanol, 50% methanol, 25% methanol and aqueous) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with eight layers of muslin cloth; centrifuged at 5000 rpm for 10 min, the supernatant was collected and the solvents were evaporated using rotary vacuum evaporator (Equitron, India) to dryness. The extract was stored at 4ºC in air tight bottles. The residues were weighed to obtain the extraction yield.

Antimicrobial activity

Microorganisms tested

Studied microorganisms were obtained from National Chemical Laboratory (NCL), Pune, India. The microorganisms were maintained at 4°C. The Gram positive bacteria studied were *Corynebacterium rubrum* ATCC14898 (*CR*), *Staphylococcus albus* NCIM 2178 (*SAL*), *Staphylococcus aureus* ATCC29737 (*SA*), *Listeria monocytogenes* ATCC19112 (*LM*), *Micrococcus flavus* ATCC10240 (*MF*), Gram negative bacteria were *Salmonella typhimurium* ATCC23564 (*ST*), *Escherichia coli* NCIM2931 (*EC*), *Proteus mirabilis* NCIM2241 (*PM*), *Citrobacter frundii* NCIM2489 (*CF*), *Enterobacter aerogenes* ATCC13048 (*EA*), and Fungi were *Candida epicola* NCIM3367 (*CE*), *Candida albicans* ATCC2091 (*CA*), *Candida glabrata* NCIM3448 (*CG*), *Cryptococcus neoformans* NCIM3542 (*CN*). The organisms were maintained on nutrient agar and MGYP medium (Hi Media, India) for bacteria and fungi respectively, at 4°C and sub-cultured before use. The microorganisms studied are clinically important ones causing several infections.

Antibiotics used in this study

The antibiotics ampicillin ($AMP^{10\mu g/disc}$), chloramphenicol ($C^{30\mu g/disc}$), tetracyclin ($T30^{\mu g/disc}$) and Ceftazidime were purchased from Hi-Media Laboratory Pvt. Ltd., (Mumbai, India). Chloramphenicol and Ceftazidime were used for MIC and MBC.

Preparation of bacterial inocula

The inocula of the test organisms were prepared using the colony suspension method[17]. Colonies picked from 24 h old cultures grown on nutrient agar were used to make suspension of the test organisms in saline solution to give an optical density of approximately 0.1 at 600nm. The suspension was then diluted 1:100 by transfer of 0.1 ml of the bacterial suspension to 9.9 ml of sterile nutrient broth before use to yield 6×10^5 CFU ml^{-1}.

Agar well diffusion method

In vitro antimicrobial activity of the different solvent extracts was studied against pathogenic microbial strains by the agar well diffusion method[18]. Muller Hinton No. 2 / Sabouraud dextrose agar (Hi-media) was used for the antibacterial and antifungal susceptibility test respectively. The different solvent extracts were diluted in 100% DMSO at the concentration of 20 mg ml^{-1}. The antimicrobial activity of crude extracts was studied against 15 microorganisms. The Muller Hinton agar / Sabouraud dextrose agar was melted and cooled to 48-50°C and a standardized inoculum (1.5×10^8 CFU ml^{-1}, 0.5 McFarland) was then added aseptically to the molten agar and poured into sterile Petri dishes to give a solid plate. Wells were prepared in the seeded agar

plates. The test compound (100 µl) was introduced in the well (8.5 mm). The plates of bacteria and fungi were incubated over night at 37°C and 28°C for 24 h and 48 h respectively. DMSO was used as negative control. The microbial growth was determined by measuring the diameter of the zone of inhibition and the mean values are presented with ± S.E.M.

Preparation of the extracts and/or antibiotic for MIC and MBC study

Two-fold serial dilutions using 100% DMSO were carried out from the 1250 µg ml^{-1} stock plant extract to make 6 test concentrations ranging from 39 to 1250 µg ml^{-1} for each solvent extracts. Two-fold dilutions of chloramphenicol and Ceftazidime (1 to 32 µg ml^{-1}) were used as a positive control.

Determination of Minimum Inhibitory Concentration (MIC)

The MICs were determined only for the test organism that had shown > 15 mm zone of inhibition to the crude extracts. Micro broth dilution method, with sterile flat bottom 96 well micro test plates (Tarsons Products Pvt. Ltd.), was performed to evaluate MIC of the plant extracts[19]. A 150 µl volume of Muller-Hinton broth was introduced into all the 96 wells and 20 µl of the varying concentrations of the extract were added in decreasing order along with 30 µl of the test organism suspension. A final volume of 200 µl was achieved in each well (150 µl Muller-Hinton broth, 30 µl of the test organism suspension, and 20 µl plant extract/antibiotic). Three control wells were maintained for each test batch. The positive control (antibiotic, Muller-Hinton broth and test organism) and sterility control (Muller-Hinton broth and DMSO) and organism control (Muller-Hinton broth, test organism and DMSO). Plates were then incubated at 37°C for 24 h overnight. Experiments were performed in duplicate. After incubation, 40 µl of 2-(4-Iodo phenyl)-3-(4-nitro phenyl)5-phenyltetrazolium chloride (I.N.T.) (Himedia, India) solution (0.2 mg ml^{-1}) dissolved in sterile distilled water was added to each well. The plates were incubated for a further 30 min, and estimated visually for any change in color in to pink indicating reduction of the dye due to bacterial growth. The highest dilution (lowest concentration) that remained clear corresponded to the MIC.

Minimum Bactericidal Concentration (MBC)

The MBC was determined by samples from all wells showing no growth as well as sample from the lowest concentration showing growth in the MIC assay, were subculture on freshly prepared nutrient agar. Plates were incubated for 24h at 37°C. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC[20].

Statistical analysis

All experiments were repeated at least three times. Results are reported as Mean ± S.E.M. (Standard Error of Mean).

RESULTS AND DISCUSSION

Extractive yield

Traditionally, water is used for extraction but since many secondary metabolites are not fully extracted in water, organic solvents are used. However, it was thought of interest to use pure water, pure methanol and hydro alcoholic extracts. Hence, in the present study 100% methanol, 75% methanol, 50% methanol, 25% methanol and aqueous extracts were made.

The extractive yield varied among different parts and among the different hydro alcoholic solvents used (Fig. 2). In all the parts, 25% methanol extract showed

highest extractive yield while hexane showed minimum extractive yield than the other hydro alcoholic solvent extracts. In hexane extracts, it can be ranked from high to low in the following order peel (1.305) > aerial part (0.83) > pulp (0.755). In 100% methanol extracts, it can be ranked from high to low in the following order pulp (21.55) > peel (16.82) > aerial part (5.51). In 75% methanol extracts, it can be ranked from high to low in the following order pulp (34.75) > peel (22.16) > aerial part (12.26). In 50% methanol extracts, it can be ranked from high to low in the following order pulp (35.56) > peel (22.15) > aerial part (16.59). In 25% methanol extracts, it can be ranked from high to low in the following order pulp (38.97) > peel (24.46) > aerial part (16.49). In aqueous extracts, it can be ranked from high to low in the following order pulp (37.36) > peel (22.48) > aerial part (16.38). Among all the parts, pulp showed better extractive yield than other parts (Fig. 2c). The extraction yield depends on type of solvents, time and temperature of extraction, as well as the chemical nature of the sample. Under the same time and temperature conditions, the solvent used and the chemical property of sample are the two most important factors[21].

Antimicrobial screening

The use of medicinal plants play a vital role in covering the basic health needs and also offer a new source of antibacterial and antifungal agents with significant activity against pathogenic microorganisms. Antimicrobial activity of different parts (aerial part, peel and pulp) of *L. siceraria* was evaluated against five Gram positive bacteria, five Gram negative bacteria and four fungi. The antimicrobial activity was determined by measuring the zone of inhibition. All the extracts showed different level of antimicrobial activity against the selected bacteria and fungi.

The antimicrobial activity of different solvent extracts of aerial part of *L. siceraria* is shown in Fig. 3. The antibacterial activity of aerial part against Gram positive bacteria is shown in Fig. 3a. All the extracts showed activity against *M. flavus* and *L. monocytogens* except hexane and water extract respectively. The highest activity was in 75% methanol extract followed by 100% methanol extract against *M. flavus*. The antibacterial activity of aerial part against Gram negative bacteria is shown in Fig. 2b. All the extracts showed activity against tested Gram negative bacteria; 75% methanol extract showed highest activity against *P. mirabilis*. The antifungal activity of aerial part against four fungi is shown in Fig. 3c. All the extracts showed activity against all the fungi except hexane extract against *C. epicola*. All the extracts of aerial part inhibited 63.33% Gram positive bacteria, 93.33% Gram negative bacteria and 95.83% fungi.

The antimicrobial activity of different solvent extracts of peel of *L. siceraria* is shown in Fig. 4. The antibacterial activity of peel against Gram positive bacteria is shown in Fig. 4a. The hexane extract showed activity against all the Gram positive bacteria and highest was against *M. flavus*. *S. albus* was more resistant towards all the extracts. The antibacterial activity of peel against Gram negative bacteria is shown in Fig. 4b. All the extracts showed activity against *E. coli* and *E.* aerogens. Aqueous extract showed highest activity against *S. typhimurium*. The antifungal activity of peel against four fungi is shown in Fig. 4c. All the extracts showed activity against all the fungi. All the extracts of peel inhibited 33.33% Gram positive bacteria, 86.66% Gram negative bacteria and 100% fungi.

The antimicrobial activity of different solvent extracts of pulp of *L. siceraria* is shown in Fig. 5. The antibacterial activity of pulp against Gram

positive bacteria is shown in Fig. 5a. All the extracts showed activity against *M. flavus* and *S. albus*. The highest activity was in aqueous extract against *S. aureus* followed by *M. flavus*. The antibacterial activity of pulp against gram negative bacteria is shown in Fig. 5b. All the extracts showed activity against *C. freundii*, *E. aerogens* and *E. coli*. Hexane extract showed highest activity against *S. typhimurium*. The antifungal activity of pulp against four fungi is shown in Fig. 5c. All the extracts showed activity against all fungi except hexane extract against *C. epicola*. All the extracts of pulp inhibited 63.33% Gram positive bacteria, 73.33% inhibition against Gram negative bacteria and 95.83% inhibition against fungi.

All the 18 extracts were compared with 5 standard antibiotics. These antibiotics were tested against 14 medically important microbial strains, the results of which are presented in Table 1. The antimicrobial activity of some of the solvent extracts was comparable with that of standard antibiotics. From the above results it can be concluded, that *M. flavus* was the most susceptible Gram positive bacteria. Amongst the Gram negative bacteria, *E. aerogenes* and *E. coli* were the susceptible strain. All the fungal strains tested were susceptible.

In the present work, all the extracts showed better antifungal activity than antibacterial activity. Similar reports are reported by other workers[22]. Amongst the Gram positive and negative bacterial strains screened, Gram negative bacteria were more susceptible than Gram positive bacteria. Similar results are also reported in *Citrus bergamia* peel[23], *Woodfordia fruticosa* flower[16] leaves of *Gymnema sylvestre* and *Eclipta prostate*[24]. This is in contrast to the general belief that Gram positive bacteria are more susceptible to herbal drugs than Gram negative bacteria[25-29].

In recent years, several diseases and microbial infections such as respiratory infections, bacterial meningitis, sexually transmitted as well as hospital acquired infections, particularly those caused by the members of the family Enterobacteriaceae have shown considerable resistance to a number of antimicrobial agents, such as penicillin, ampicillin, and flouroquinolones among many others[30,31]. Therefore, there is always a dire need to search for agents preferably natural agents that are able to curb at least some of the infection causing microorganisms. The results of the present study showed promising results towards Gram negative bacteria and fungi.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of the antimicrobial agent that will inhibit the visible growth of a microorganism after overnight incubation**.** The minimum bactericidal concentration (MBC) is interpreted as the lowest concentration that can completely remove the microorganisms. The MIC and MBC of different solvent extracts of different parts of *L. siceraria* and standard antibiotics are shown in Tables 2-4. Inhibitory effects of bacterial growth by the extracts from different parts were in the range from <312 to > 1250 µg ml^{-1} expressed as MIC values and in the range from 1250 to >1250 µg ml^{-1} expressed as MBC values. Inhibitory effects of bacterial growth by the standard antibiotics were in the range from 2 to > 32 µg ml^{-1} expressed as MIC values and in the range from 8 to >32 µg ml^{-1} expressed as MBC values.

The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of different solvent extracts of aerial part of *L. siceraria* and standard antibiotics are shown in Table 2. 100% methanol showed least

MIC and MBC values i.e. 625 µg ml^{-1} and >1250 µg ml^{-1} respectively against *M. flavus*. However 100% methanol showed MIC and MBC values 1250 µg ml^{-1} and >1250 µg ml^{-1} respectively against *S. typhimurium*. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of different solvent extracts of *L. siceraria* peel and standard antibiotics are shown in Table 3. Hexane extract showed least MIC and MBC values i.e. 312 µg ml^{-1} and 1250 µg ml^{-1} respectively against *M. flavus*. 75% methanol and 50% methanol showed similar MIC and MBC values against *S. aureus*. However all the extracts showed MIC and MBC values >1250 µg ml^{-1} against *S. typhimurium*. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of different solvent extracts of *L. siceraria* pulp and standard antibiotics are shown in Table 4. 25% methanol showed MIC and MBC values were 625 µg ml^{-1} and >1250 µg ml^{-1} respectively against *M. flavus*. However all the extracts showed MIC and MBC values >1250 µg ml^{-1} against *S. typhimurium P. mirabilis* and *S. aureus*.

The present study showed that different parts of *L. siceraria* extracts possessed *in vitro* antimicrobial property against infectious diseases caused by microorganisms. The peel, an agro waste which is normally thrown away possesses antimicrobial property, which is noteworthy. However, it is necessary to carry out a bioassay guided fractionation of the extract in a bid to isolate and identify the compounds responsible for the antimicrobial activity.

CONCLUSIONS

The different solvent extracts showed better antifungal activity than antibacterial activity. Gram negative bacteria were more susceptible towards all the extracts than Gram positive bacteria. *M. flavus* was the most susceptible Gram positive bacteria while *C. fruendi, E. aerogenes* and *E. coli* were most susceptible Gram negative bacteria. All the tested fungi were susceptible to all the extracts. The lowest MIC and MBC values were observed in hexane extracts of peel i.e. 312 and 1250 µg/ml. The results of this study may be useful as potential bio-control agents and afford efficient and safe antimicrobials which will certainly contribute to the ongoing search for new antimicrobial agents to fight against infectious diseases caused by antibiotic resistant strains.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

ACKNOWLEDGMENTS

The authors thank Prof. S. P. Singh, Head, Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India for providing research facilities.

REFERENCES

1. Gibbons S. Anti-staphylococcal plant natural products. Nat Prod Res 2004; 21:263-277.
2. Somchit MN, Reezal I, Nur EI, Mutalib AR. *In vitro* antimicrobial activity of ethanol and water extract of *Cassia alata. J Ethnopharmacol* 2003; 84:1-4.
3. Fazly-Bazzaz, Karamadin MK, Shokooheizadeh HR. *In vitro* antibacterial activity of *Rheum ribes* extract obtained from various plant parts against clinical isolates of Gram-negative pathogens. *Iranian J Pharm* 2005; 2:87-91.
4. Wagner H, Merzenich U. Synergy research: Approaching a new generation of phyto pharmaceuticals. *Phytomedicine* 2009; 16:97-110.

5. Zampinia IC, Villena J, Salvaa, Herrera SM, Islac MI, Alvarez S (2012). Potentiality of standardized extract and isolated flavonoids from *Zuccagnia punctata* for the treatment of respiratory infections by *Streptococcus pneumonia*, *in vitro* and *in vivo* studies. *J Ethnopharmacol* 2012; 140:287–292.
6. Ekundayo FO, Adeboye CA and Ekundayo EA. Antimicrobial activities and phytochemical screening of pignut (*Jatrophas curcas* Linn.) on some pathogenic bacteria. *J Med Plants Res* 2011; 5:1261-1264.
7. Chanda S, Kaneria M. Optimization of conditions for the extraction of antioxidants from leaves of *Syzygium cumini* L. using different solvents. *Food Anal Methods* 2012; 5:332-338.
8. Chanda S, Kaneria M, Baravalia Y. Antioxidant and antimicrobial properties of various polar solvent extracts of stem and leaves of four *Cassia* species. *Afr J Biotechnol* 2012; 11:2490-2503.
9. Khatoon R, Jahan N, Ahmad S, Shahzad A. *In vitro* evaluation of antifungal activity of aerial parts of medicinal plants Balanites aegyptiaca Del. and Spilanthes acmella Murr. *J Appl Pharmaceut Sci* 2014; 4:123-127.
10. Anjaria J, Parabia M, Dwivedi S. Ethanovet Heritage Indian Ethnoveterinary Medicine – An Overview, pp 420. Pathik Enterprise, Ahmedabad, India, 2012.
11. Srivastava J, Lambert J, Vietmeyer N. Medicinal plants: An expanding role in development. World Bank Technical Paper 320, 1996.
12. Ghule, BV, Ghante MH, Saoji AN, Yeole PG. Hypolipidemic and antihyperlipidemic effects of *Lagenaria siceraria* (Mol.) fruit extract. *Indian J Expt Biol* 2006; 44:905-909.
13. Ghule, BV, Ghante MH, Saoji AN, Yeole PG. Diuretic activity of *Lagenaria siceraria* fruit extract in rats. *Indian J Pharm Sci* 2007; 69:817-819.
14. Hassanpour f, M, Bodhankar SL, Dikshit M. Cardioprotective activity of fruit of *Lagenaria siceraria* (Molina.) standly on doxorubicin induced cardiotoxicity in rats. *J Pharmacol* 2008; 4:466-471.
15. Rakholiya K, Kaneria M, Chanda S. Vegetable and fruit peels as a novel source of antioxidants. *J Med Plants Res* 2011; 5:63-71.
16. Parekh J, Chanda S. *In vitro* antibacterial activity of the crude methanol extract of *woodfordia fruticosa* kurz. Flower (Lythraceae) *Braz J Microbiol* 2007a; 38:204-207.
17. European Committee for Antimicrobial Susceptibility Testing (EUCAST). Determination of minimum inhibitory concentrations (MICs) of antibacterial agents by broth dilution, *Clin Micro Inf* 2003; 9:1-7.
18. Perez C, Paul M, Bazerque P. An antibiotic assay by the agar well diffusion method. Acta Biol Med Expt 1990; 15: 113-115.
19. Sampaio FC, Pereira Mdo S, Dias CS, Costa VC, Conde NC, Buzalaf MA. *In vitro* antimicrobial activity of *Caesalpinia ferrea* Martius fruits against oral pathogens. *J Ethnopharmacol* 2009; 124: 289-294.
20. Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in South-West Nigerian unorthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity, *BMC Complement. Altern Med* 2005; 5:6
21. Shimada K, Fujikawa K, YaharaK, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil incyclodextrin emulsion. *J. Agric. Food Chem* 192; 40: 945-948.
22. Adeniyi BA and Odufowora RO. *Invitro* antimicrobial properties of *Aspilla africana* (compositae). *Afr J Biomed Res* 2000; 3:167-170.
23. Mandalari G, Bennett RN, Bisignano G, Trombetta D, Saija A, Faulds CB, Gasson MJ, Narbad A. Antimicrobial activity of flavonoids extracted from bergamot (*Citrus bergamia* Risso) peel, a byproduct of the essential oil industry. *J Appl Microbiol* 2007; 103:2056-2064.
24. Khanna V, Kannabiran K. Antimicrobial activity of saponin fractions of the leaves of *Gymnema sylvestre* and *Eclipta prostate*. *World J Microbiol Biotechnol* 2008; 24:2737-2740

25. Parekh J, Chanda S. *In vitro* antimicrobial activities of extracts of *launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). *Afr J Biomed Res* 2006; 9:89-93.
26. Parekh J, Chanda S. *In vitro* antimicrobial activity of *Trapa natans* L. fruit rind extracted in different solvents. *Afr J Biotechnol* 2007b; 6:766-770.
27. Li A, Zhu Y, He X, Tian X, Xu L, Ni W, Jiang P. Evaluation of antimicrobial activity of certain Chinese plants used in folkloric medicine. *World J Microbiol Biotechnol* 2008; 24:569-572
28. Fernandez-Agullo A, Pereira E, Freire MS, Valentao P, Andrade PB, Gonzalez-Alvarez J and Pereira JA (2013). Influence of solvent on the antioxidant and antimicrobial properties of walnut (Juglans regia L.) green husk extracts. *Indus Crops Prod* 2013; 42:126-132.
29. Bhat RS and Al-Daihan S. Phytochemical constituents and antibacterial activity of some green leafy vegetables. *Asian Pac J Trop Biomed* 2014; 4:189-193.
30. Okeke IN, Aboderin OA, Byarugaba DK, Ojo KK and Opintan JA. Growing problem of multidrug resistant enteric pathogens in *Africa. Emerg Infect Dis Perspect* 2007; 13:393-396.
31. Mwambete KD. The *in vitro* antimicrobial activity of fruit and leaf crude extracts of *Momordica charantia*, A Tanzania medicinal plant. *Afr Health Sci* 2009; 9:34-39.

Table 1. Antimicrobial activity using standard antibiotics

Antibiotic name	Conc.	Gram positive					Gram negative					Fungi			
		LM	*SA*	*MF*	*SA*	*CR*	*ST*	*PM*	*EC*	*EA*	*CF*	*CE*	*CA*	*CG*	*CN*
Ampicilin	10 µg	0	0	0	23	25	0	9	0	0	0	ND	ND	ND	ND
Tetracycline	30 µg	0	21	0	22	0	21	20	16	25	20	ND	ND	ND	ND
Chloramphenicol	30 µg	0	26	0	18	15	28	0	19	23	ND	ND	ND	ND	ND
Nystatin	100 units	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	22	18	18	22
Amphotericin	100 units	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	16	10	10	11

ND = Not done

SA: Staphylococcus aureus, SAL: Staphylococcus albus, CR: Corynebacterium rubrum, LM: Listeria monocytogenes,MF: Micrococcus flavus, ST: Salmonella typhimurium, EA: Enterobacter aerogenes, CR: Citrobacter frundii, EC: Escherichia coli, PM: Proteus mirabilis, CE: Candida epicola, CA: Candida s albicans, CN: Cryptococcus neoformans, CG: Candida s glabrata.

Table 2. MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of different solvent extracts of aerial part of *L. siceraria*

No.	Extracts	*SA*[1]		*MF*[1]		*ST*[2]		*PM*[2]	
		MIC*	MBC*	MIC*	MBC*	MIC*	MBC*	MIC*	MBC*
1	HE	-	-	-	-	-	-	-	-
2	100% ME	-	-	625	>1250	1250	>1250	-	-
3	75% ME	-	-	1250	>1250	-	-	>1250	>1250
4	50% ME	-	-	-	-	-	-	-	-
5	25% ME	-	-	-	-	-	-	-	
6	AQ	-	-	>1250	>1250	-	-	-	-
7	CH	2	>32	4	16	4	>32	8	>32
8	CF	4	>32	16	32	<1	8	>32	>32

*: Values are expressed in µg ml^{-1}, -: Not tested, 1: Gram positive bacteria, 2: Gram negative bacteria

Table 3. MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of different solvent extracts of *L. siceraria* peel

No.	Extracts	*SA*[1]		*MF*[1]		*ST*[2]		*PM*[2]	
		MIC*	MBC*	MIC*	MBC*	MIC*	MBC*	MIC*	MBC*
1	HE	-	-	312	1250	-	-	-	-
2	100% ME	-	-	-	-	>1250	>1250	-	-
3	75% ME	1250	>1250	-	-	>1250	>1250	-	-
4	50% ME	1250	>1250	-	-	>1250	>1250	-	-
5	25% ME	-	-	-	-			-	-
6	AQ	-	-	1250	>1250	>1250	>1250	-	-
7	CH	2	>32	4	16	4	>32	8	>32
8	CF	4	>32	16	32	<1	8	>32	>32

*: Values are expressed in µg ml^{-1}, -: Not tested, 1: Gram positive bacteria, 2: Gram negative bacteria

Table 4. MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) values of different solvent extracts of *L. siceraria* pulp

No.	Extracts	*SA*[1]		*MF*[1]		*ST*[2]		*PM*[2]	
		MIC*	MBC*	MIC*	MBC*	MIC*	MBC*	MIC*	MBC*
1	HE	-	-	-	-	>1250	>1250	-	-
2	100% ME	-	-	-	-	-	-	-	-
3	75% ME	-	-	-	-	-	-	-	-
4	50% ME	-	-	-	-	-	-	-	-
5	25% ME	-	-	625	>1250	-	-	-	-
6	AQ	>1250	>1250	>1250	>1250	-	-	-	-
7	CH	2	>32	4	16	4	>32	8	>32
8	CF	4	>32	16	32	<1	8	>32	>32

*: Values are expressed in µg ml^{-1}, -: Not tested, 1: Gram positive bacteria, 2: Gram negative bacteria

Figure 1. Photograph of *Lagenaria siceraria* (Molina) Standl

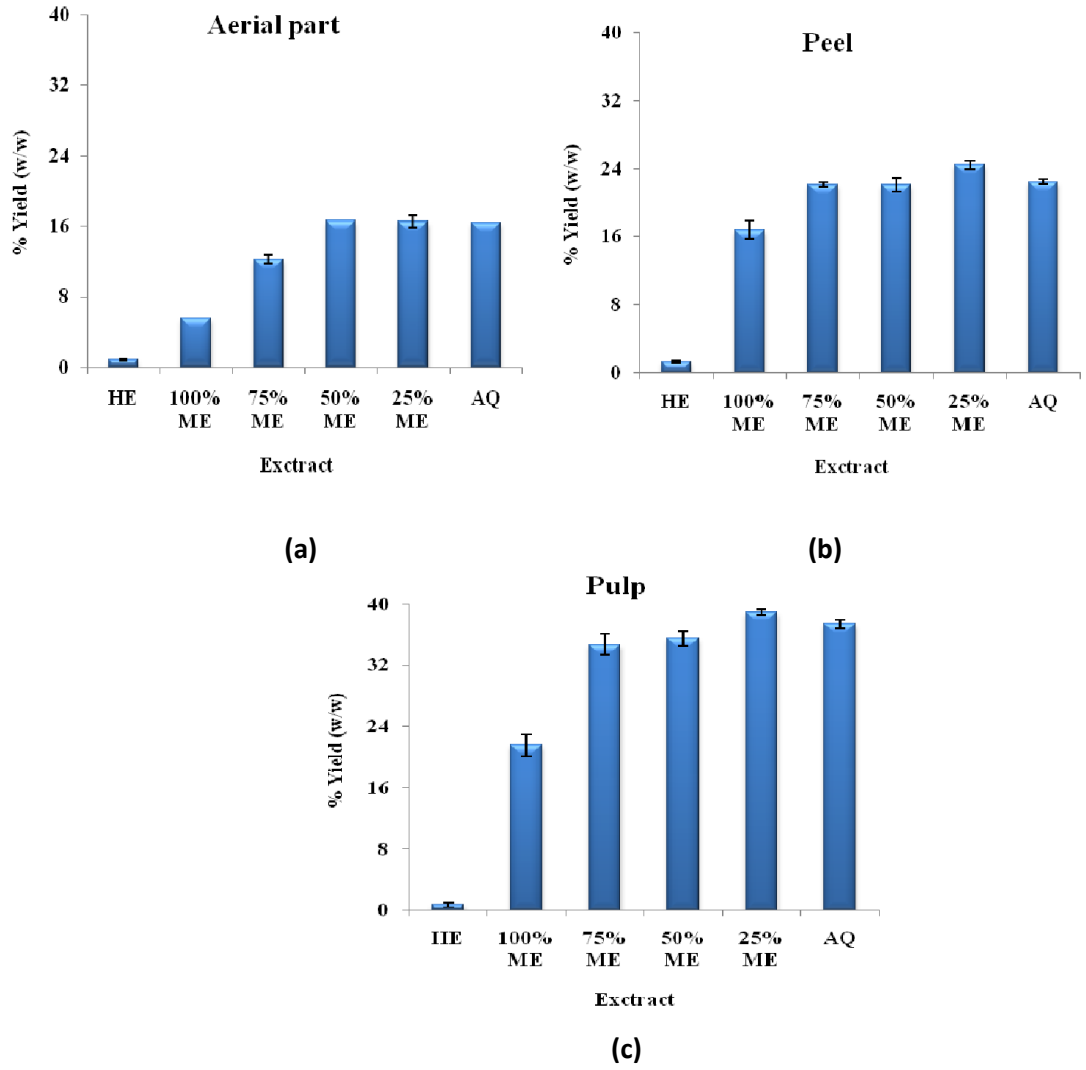

Figure 2. Percentage yield of different solvent extracts of the different parts of *L. siceraria*

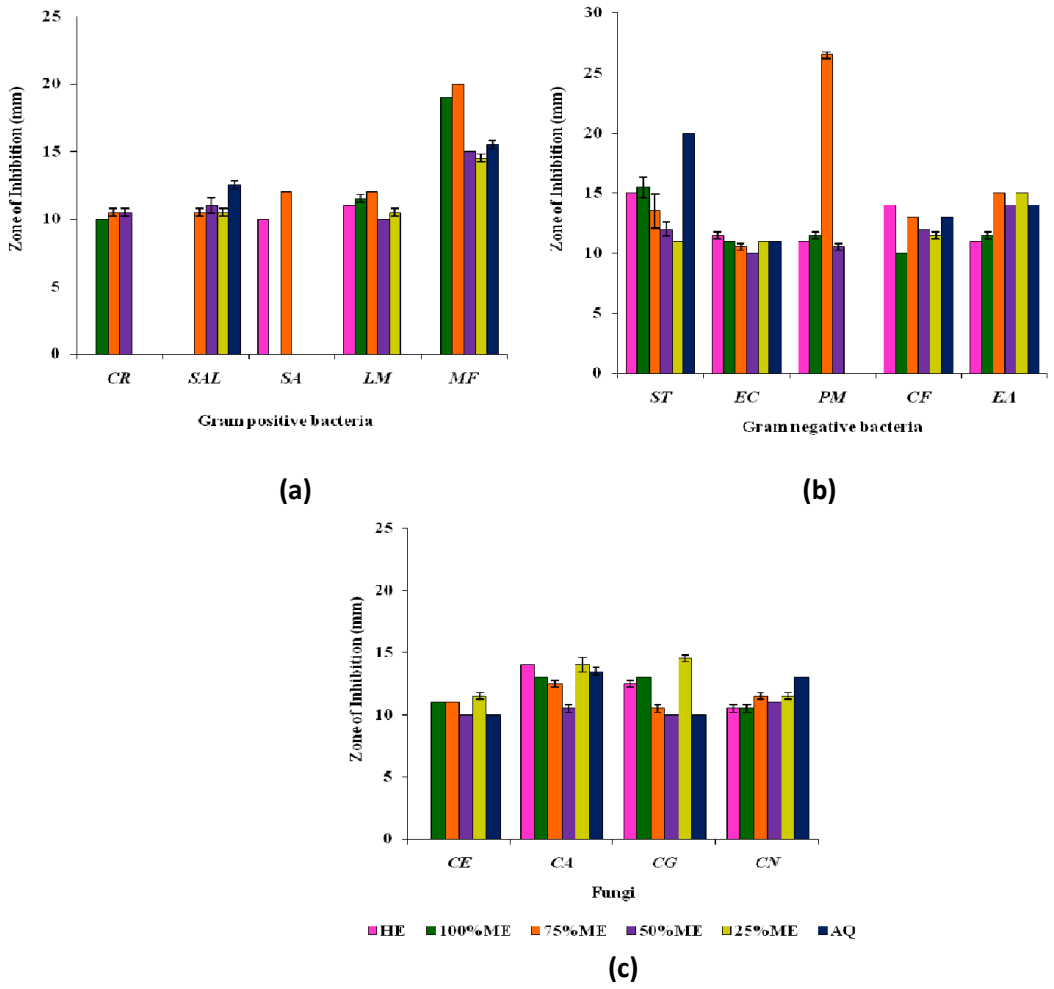

Figure 3. Antimicrobial activity of different solvent extracts of aerial part of *L. siceraria*

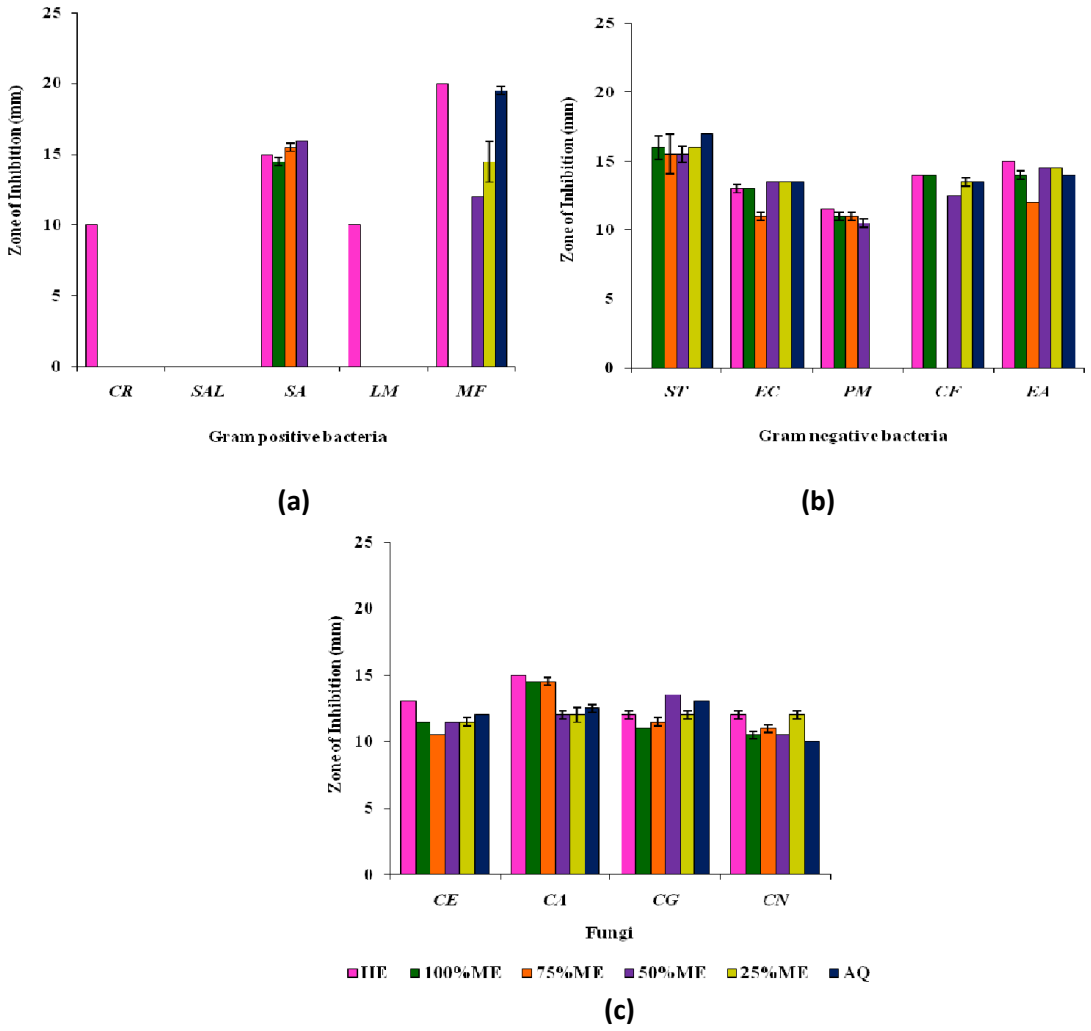

Figure 4. Antimicrobial activity of different solvent extracts of peel of *L. siceraria*

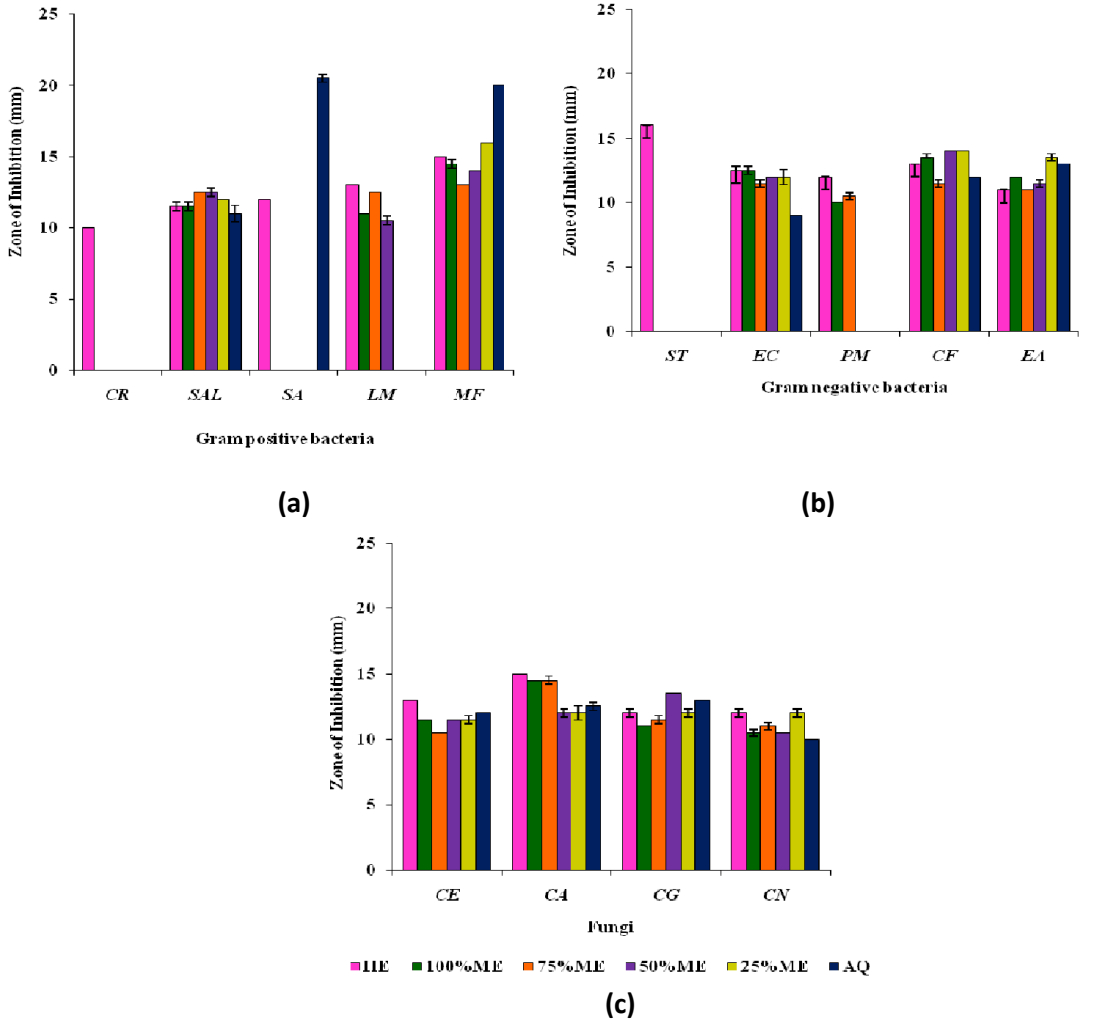

Figure 5. Antimicrobial activity of different solvent extracts of *L. siceraria* pulp