International Journal of Plant Sciences and Phytomedicines (IJPSP)
Volume 1; Issue 1; 2021; Page No. 33-50

ANTIBACTERIAL POTENTIAL AND PHYTOCHEMICAL SCREENING OF LEAVES AND ROOT EXTRACT OF *Manilkara Zapota* L. (CHIKU)

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Received Date: 30-11-2021; Accepted Date: 15-12-2021; Published Date: 14-12-2021

ABSTRACT

Manilkara zapota L. commonly known as chiku is a shrub, belongs to the family Sapotaceae. The different parts of the plant such as the leaves, bark and seeds have been extensively studied for antibacterial activity. The current study was aimed at estimation of antibacterial potential of methanolic, ethanolic and aqueous extracts of *Manilkara zapota* prepared in various concentrations *i.e*. 25 mg/ml, 50mg/ml and 75mg/ml against six bacterial strains *i.e*. *Staphylococcus aureus, Escherichia coli (1), Escherichia coli (2), Shigella sonnei, Streptococcus pyogenes* and *Neisseria gonorrhoeae* by well diffusion method. *Staphylococcus aureus* showed more inhibition to methanolic and ethanolic extracts of all three plants and plants extracts remained almost ineffective against *Shigella sonnei*. Phytochemical screenings confirmed the presence of saponins, phenols, alkaloids and flavonoids in *M. zapota.* The antimicrobial potential exhibited by *M. zapota* validates its traditional therapeutic uses. Various phytochemicals from the selected medicinal plant yield antibacterial agents that support the capabilities of these plants as major component in possible antibacterial formulation.

INTRODUCTION

Medicinal plants are used as innate sources for the handling of different ailments from the ancient times. According to World Health Organization (WHO), about 20,000 plant species with therapeutic properties are reported and majority of the world population depends on medicines extracted from these plants for their primary health care needs (Gullece *et al*., 2006; Maregesi *et al*., 2008; Vashist and Jindal, 2012). For many years, microbial infections in humans have produced significant public health problems in developing and developed countries (Anonymous,

2004). The main common pathogenic organisms are viruses, bacteria, fungi, parasitic worms and mostly horrible diseases are caused by bacteria throughout the world (Zhang *et al*., 2006; Paterson, 2008). Antibiotics are massively used to treat bacterial infections, and to reduce the burden of harmful diseases (Nananda and Connor, 1987). Antibiotics have adverse effects such as immune inhibition and allergic reaction. It has forced scientists throughout the world to search for alternatives of commercial antibiotics (Yala *et al*., 2001; Anisimov and Amoako, 2006; Abiramasundari *et al*., 2011; Bibi *et al*., 2011). An example of such approach is the utilization of natural sources, especially plants that have medicinal potentials (Mahmoodally *et al*., 2010; Savoia, 2012). It is reported that many plant have antibacterial, antifungal and insecticidal properties (Satish *et al*., 1999; Bouamama *et al*., 2006; Ergene *et al*., 2006; Kiran and Raveesha, 2006; Okigbo and Ogbonnaya, 2006; Shariff *et al*., 2006; Bhalodia *et al*., 2011; Ahmed *et al*., 2012). Due to availability of chemical diversity in the plant kingdom, they provide opportunities for new drug (Cos *et al*., 2006; Maregesi *et al*., 2008).

Manilkara zapota, commonly known as chiku is a shrub, belongs to the family Sapotaceae. It is also called by other common names such as sapota plum, sapodilla or prickly pear. Chiku grows in warm and sunny location. Its original home is Central America but the distribution extends to Florida, Philippines, Ceylon, India and Pakistan. It is mainly cultivated in most parts of world for its fruit value, while in some countries such as South-East Mexico, It is grown on commercial level for the production of chickle which is a gum like substance and mainly used for production of chewing gum (Bharath, 2014; Gupta *et al*., 1981). The different parts of the plant such as the leaves (Osman *et al*., 2011) bark (Islam *et al*., 2013) and seeds (Kothari *et al*., 2010) have been extensively studied for antimicrobial activity. Seeds have been proved to have diuretic, antibacterial and anthelminthic activity (Vijay and Sriram, 2010; Biren *et al*., 2012; Yashvanthkumar *et al*., 2012). Bark extract has been evaluated for antimicrobial and anticancer activities and contains the water soluble tuberculostatic agent (Osman *et al*., 2011).

Major objectives of the present study were following:

- Assessment of the antimicrobial potential of ethanol, methanol and aqueous extracts of *Manilkara zapota* six selected bacterial strains.

- Phytochemical screening of *Manilkara zapota* (leaves and bark extracts) for different phytochemicals *e.g.* alkaloids, flavonoids, saponins and tannins etc.

MATERIALS AND METHODS

Collection of Plant Materials

Fresh plants of *Manilkara zapota* were taken from rural areas of Gujrat and Kharian, Pakistan. Plants specimens were made for further reference by drying, pressing and mounting on herbarium sheets and deposited for the herbarium in the Department of Botany, University of Gujrat, Pakistan.

Preparation of Plant Samples

The Plant material of *Manilkara zapota* (leaves+ bark) were dried in air at room temperature (37°C) for 2 weeks, after which they were chopped into pieces and ground to a fine powder by using kitchen grinder. The samples were kept in air tight bags in dark until extraction.

Extraction of Plant Materials

Plants in powder form were extracted by shaking with three different solvents *i.e.* methanol, water and ethanol (10 grams plant powder and 100ml solvent) on incubator shaker at 220 revolution per minute at 28°C for 24 hours. Upper layer was filtered and centrifuged at 10,000 rpm for 10 minutes at 28°C. The extracts then placed in rotary evaporator to evaporate solvents at 40 °C, 41°C and 60°C for ethanol, methanol and water respectively. After evaporation, extracts were dissolved in Dimethyl Sulfoxide (DMSO) to prepare different concentrations (Nair and Chanda, 2007; Parekh and Chanda, 2007; Alipour and Khanmohammadi, 2011).

Antimicrobial Test

Manilkara zapota extracts were assessed for their antibacterial activity against the selected bacterial strains.

Preparation of media

A) **Luria Bertani (LB) Liquid**

The LB liquid medium (for one liter of LB liquid) was prepared by adding 10 g peptone, 5 g yeast extract and 10 g NaCl to 950 ml of distilled water. Medium was autoclaved for 20 minutes and stored at 4°C under aseptic conditions for further use.

B) Luria Bertani (LB) Agar

The LB liquid was prepared by the method described above and 15 g agar per liter was added to the mixture prior to autoclaving. Media was allowed to cool and poured aseptically into petri plates to make it harder and stored inverted at 4 °C under sterile conditions until further use.

Bacterial strains used

Following bacterial strains were taken from Department of biochemistry, University of Gujrat, Gujrat and used as test organisms.

S. No.	Bacterial strains	Accession Number
1	*Escherichia coli 1*	ATCC25922
2	*Escherichia coli 2*	ATCC15224
3	*Neisseria gonorrhoeae*	4C11
4	*Streptococcus pyogenes*	TC-11- 2
5	*Shigella sonnei*	B -B – 8
6	*Staphylococcus aureus*	25923

Culturing of Bacterial Strains

Culturing of the selected bacterial strains was done on LB medium at 35-37 °C for about 18-24 hours. Streaking of Inoculated bacterial strains was done on LB Agar plates and incubated at 35-37 °C to get a single purified colony of targeted bacteria (Pandya *et al.*, 2011).

Agar Well Diffusion Test

Antibacterial potential of test plant sample was tested by well diffusion method of Hussain *et al.* (2010) with little changes. Plates containing LB agar medium was swabbed with sterilized

cotton wool containing 18-12 hour old strains of bacteria. Wells of 6mm were made in medium with help of glass borer. Plants extracts in different concentrations were prepared in DMSO and were poured in wells, along with this standard antibiotic was added in adjacent well to compare with the activity of plant extracts. Three readings were noted, their means were taken depicting the antibacterial potential of plant extracts (Hussain *et al.*, 2010).

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

Plant extracts that showed antibacterial activities were further used to determine the MIC, defined as the minimum concentration of antimicrobial agent that can inhibit the growth of the test pathogen. Bacterial strains were grown at 37°C for one night and diluted in nutrient broth medium. Prepared plant extracts were decanted in test tubes containing the test organism and incubated at 37°C for 24 hours, Then optical density was measured at 620nm by using Nutrient Broth medium (NB) as a control. The lowest concentration that was able to kill test organism on solid medium was minimum bacterial concentration (MBC).

Phytochemical Analysis

The presence of some basic therapeutic compounds in the plants was determined with the help of standard methods (Evans, 1996).

a) Alkaloids Test

For checking the presence of alkaloids, Plant extracts dissolved in dilute Hydrochloric acid (HCl) were filtered. Filtrates were treated with Mayer's reagent (Potassium Mercuric iodide). Yellow cream precipitates indicated the presence of alkaloids (Roopashree *et al.*, 2008).

b) Glycosides Test

Plant Extracts dissolved in dilute HCl were subjected to confirm presence of glycosides. Legal's Test: Extracts were dissolved in sodium nitroprusside and methanolic base. Pink to red colour appearance indicates the presence of glycosides (Roopashree *et al.*, 2008).

c) Saponins Test

Froth Test: Formation of foam confirmed the presence of saponins by diluting extract with distilled water and shaking in a graduated cylinder for 15 minutes (Roopashree *et al.*, 2008).

d) Tannins Test

Gelatin Test: In the extract sodium chloride (NaCl) in 1% gelatin was added. White precipitates confirmed the tannins presence (Roopashree *et al.*, 2008).

e) Flavonoids Test

For flavonoids test, 5ml of dilute ammonia (NH_3) was added to aqueous filtrate of the extract in presence of 1ml concentrated sulphuric acid (H_2SO_4). A yellow coloration indicated the presence of flavonoids (Khan *et al*., 2011).

f) Phenols Test

Extracts were treated with few drops of ferric chloride ($FeCl_2$) solution. If blue color appeares it confirmed the presence of phenols (Roopashree *et al.*, 2008).

g) Steroids Test

Plant extract was dissolved in alcohol and one ml of mixture was dissolved in 5 ml of chloroform ($CHCl_3$) which was further mixed with 5ml of concentrated sulphuric acid (H_2SO_4) along the sides of test tube. Sulphuric acid layer turned yellow and layer above turned red which was assigned as a steroid indication (Roopashree *et al.*, 2008).

Statistical Analysis

Results were subjected to Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) with a significance level of 0.05.

RESULTS

Antibacterial potential of *Manilkara zapota*

Antibacterial potential of plant extracts of *M. zapota* prepared in methanol, ethanol and aqueous solvent was measured by agar well diffusion method and zones of growth inhibition of selected bacterial strains were measured after 24 and 48 hours of incubation in three different concentrations (25mg/ml, 50 mg/ml and 75mg/ml).

(a) Antibacterial potential of *M. zapota* in methanolic extract

M. zapota (25mg in 100ml of DMSO) decreased selected bacterial strains growth after one day of incubation. *S. aureus* showed the maximum zone of inhibition (6.555±1.528), and *S. sonnei* showed the lowest zone of inhibition (2.166±0.000). *N. gonorrhoeae* and *S. pyogenes* has no inhibition zone at 25mg/ml after one day of inhibition (Fig 1). *S. aureus* showed highest zone of inhibition (6.578±0.777) and *S. Sonnei* showed the least zone of inhibition (3.000±0.000) at 50mg/ml. *S. aureus* has zone of inhibition (7.000±0.577) and *S. sonnei* has zone of inhibition (3.000±0.000) at 75mg/ml (Fig 1).

S. aureus has zone of inhibition *i.e*. 5.000±0.577 which is highest and *S. sonnei* has zone of inhibition of 2.000±0.000 which is lowest. *N. gonorrhoeae and S. pyogenes* had no any zone of inhibition at 25 mg/ml level of *M. zapota* after 2 days of incubation (Fig 2). In 50 and 75mg/ml level of chiku plant extract also maximum growth inhibition was shown by *S. aureus* and lowest by *S. sonnei.*

Antibacterial potential of *M. zapota* in ethanolic extract

M. zapota showed different zones of inhibition and have high antibacterial potential against *S. aureus* and *M. zapota extract* had least antibacterial potential against *S. pyogenes* after one day of incubation (Fig 3). *M. zapota* extract had no effect on the growth of *S. sonnei* and *E coli* (1). *S. aureus* gave the highest zones of inhibition and the least zones of inhibition were shown by *S. pyogenes and N. gonorrhoeae* at all three levels of *M. zapota* extracts after 48 hours of incubation (Fig. 4).

Antibacterial potential of *M. zapota* in aqueous extract

All selected strains of bacteria showed least zones of inhibition as compared to methanolic and ethanolic extract of *zapota*. Highest zones of inhibitions were observed on LB ager plates offered by *S. aureus and S. pyogenes* and lowest zones of inhibition were presented by *N. gonorrhoeae* at all three levels of plant extract. But *M. zapota* in aqueous extract was not effective

against *E. coli (2)*, *E. coli (1) and S. sonnei* (Fig 5). *M. zapota* in aqueous extracts has no activity against *Escherichia coli* (2), *Escherichia coli (1)* and *Shigella sonnei* and highest activity against *S. aureus and S. pyogenes* (3.000±0.100), but lowest zone of inhibition was observed in *N. gonorrhoeae* at all three levels of *M. zapota* and these were also compared with standard antibiotic (Fig 6).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Of *M. zapota* Extracts

MIC and MBC were determined of all three solvent extracts of *M. zapota* at two different hours of incubation (24 and 48) and values were recorded. Highest values were exhibited by *S. aureus* and *S. pyogenes*. MIC value of *S. aureus* was 12.75 mg/ml and MBC was 25.5 mg/ml, *S. pyogenes* showed MIC 11.33 mg/ml and MBC 23.34 mg/ml. Lowest values were shown by *E coli (2)* its MIC was 2.33 mg/ml and the value of MBC was 4.66mg/ml. *S. sonnei* and *N. gonorrhoeae* remained resistant (Fig 7). *S. aureus* has MIC value of 9.75 mg/ml and MBC value of 18.5 mg/ml. Minimum inhibitory zone (2.33mg/ml) and MBC (4.22 mg/ml) were noted for *S. pyogenes* (Fig 8). The growth of *S. aureus* and *S. pyogenes* inhibited at large extent. It showed 6.375 mg/ml value of MIC and the value of MBC was 12.75 mg/ml for the both of bacterial strains, and *N. gonorrhoeae* showed lowest zone of inhibition (MIC = 2.46mg/ml and MBC = 4.33mg/ml). *S. sonnei, E coli (1) and E coli (2)* remained resistant (Fig 9).

Qualitative Analysis of Phytochemicals

Manilkara zapota extracts were evaluated qualitatively for the presence and absence of different secondary metabolites by applying devised biochemical tests. Saponins, tannins, steroids, alkaloids, flavonoids, phenols, carbohydrates and glycosides were tested in methanolic extracts, ethanolic extract and aqueous extract of said plants. In methanolic and ethanolic extract of *M. zapota*, alkaloids, saponins, flavonoids, glycosides, sugar and phenols were present, and steroids were absent in methanolic and ethanolic extract of *M. zapota*. In aqueous extract of *M. zapota*, alkaloids, saponins, flavonoids and phenols were present and glycosides, sugar and steroids were absent.

Table 1: Phytochemical screening of different solvent extracts of *M. zapota.*

S. No.	Phytoconstituents	Water	Methanol	Ethanol
1	Glycosides	–	+	+
2	Flavonoids	+	+	+
3	Saponins	+	+	+
4	Phenols	+	+	–
5	Alkaloids	+	+	+
6	Steroids	–	–	–
7	Reducing Sugar	–	+	+
8	Tannins	–	+	+

–: No detected and +: Detected.

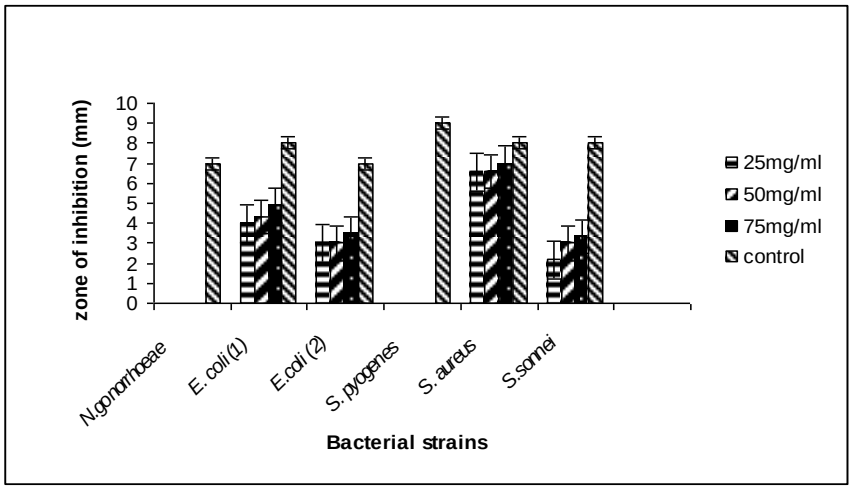

Fig. 1: Zone of inhibition of different bacterial strains after incubation of 24 hours at different concentrations of methanolic extract of *Manilkara zapota.*

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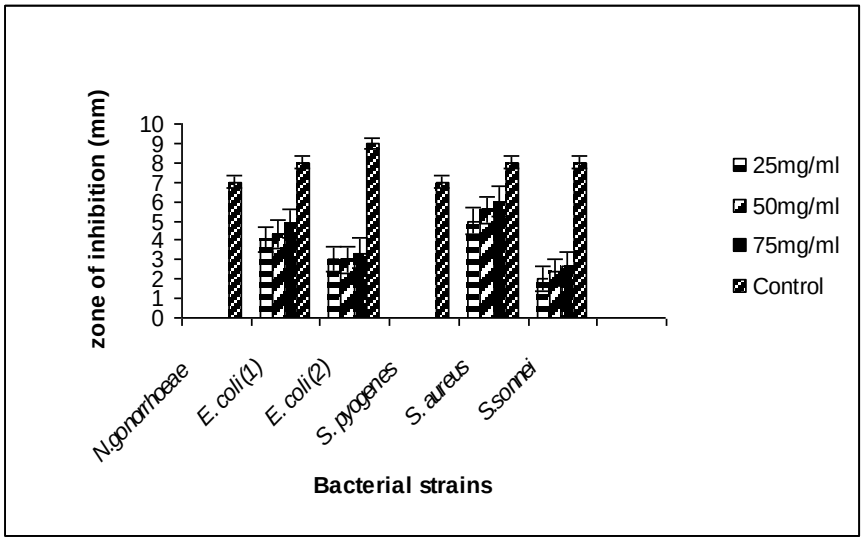

Fig. 2: Zone of inhibition of different bacterial strains after incubation of 48 hours at different concentrations of methanolic extract of *Manilkara zapota*

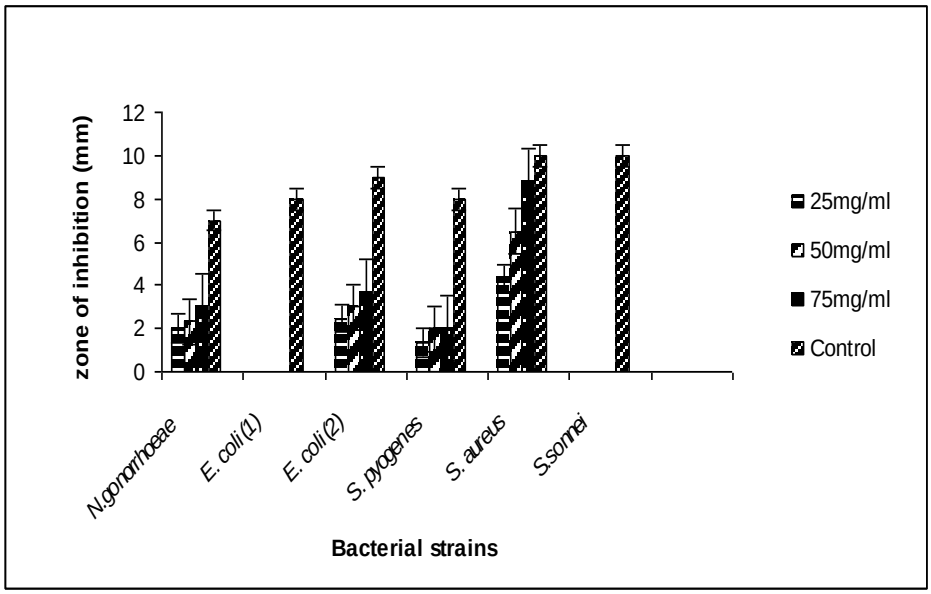

Fig. 3: Zone of inhibition of different bacterial strains after incubation of 24 hours at different concentrations of ethanolic extract of *Manilkara zapota.*

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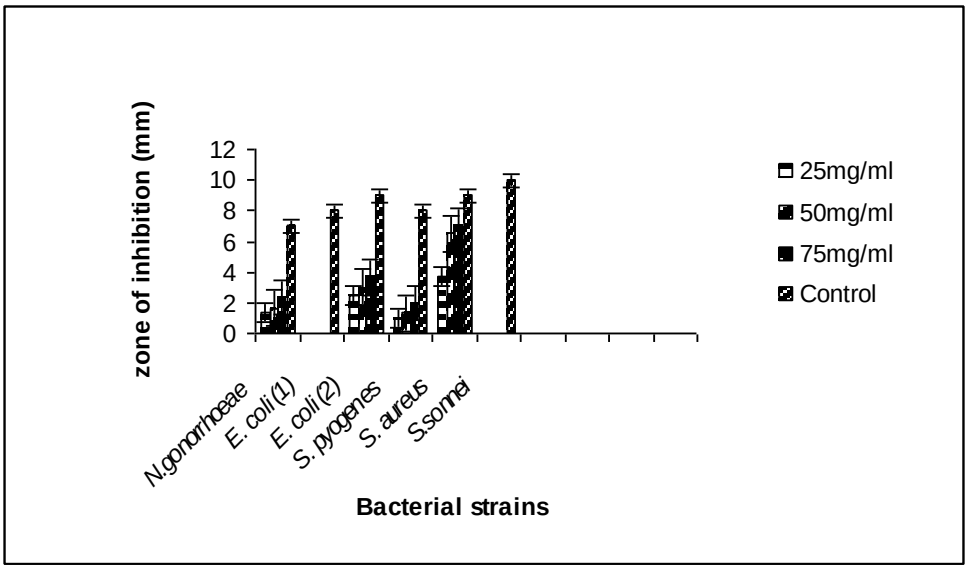

Fig. 4: Zone of inhibition of different bacterial strains after incubation of 48 hours at different concentrations of ethanolic extract of *Manilkara zapota*

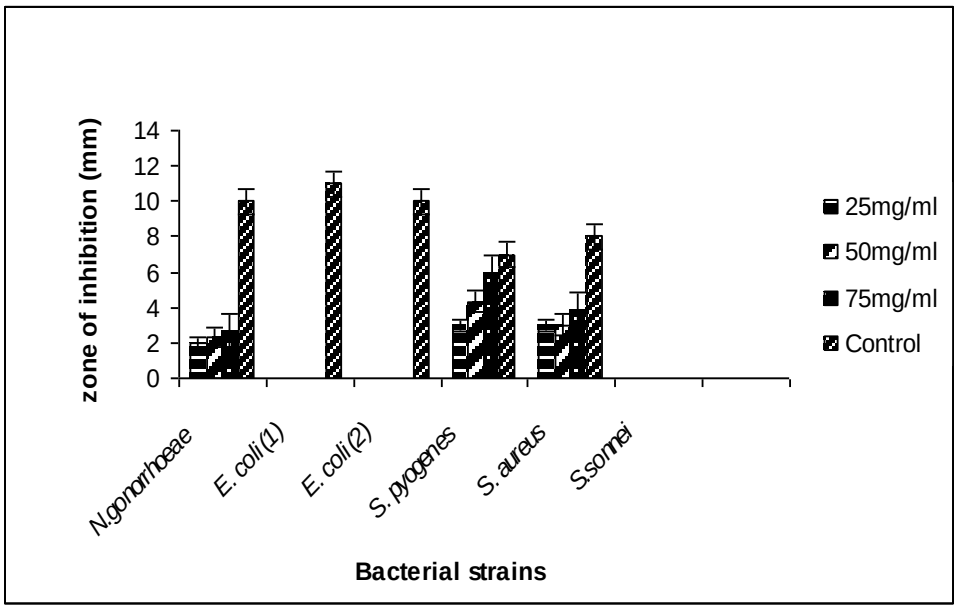

Fig. 5: Zone of inhibition of different bacterial strains after incubation of 24 hours at different concentrations of aqueous extract of *Manilkara zapota.*

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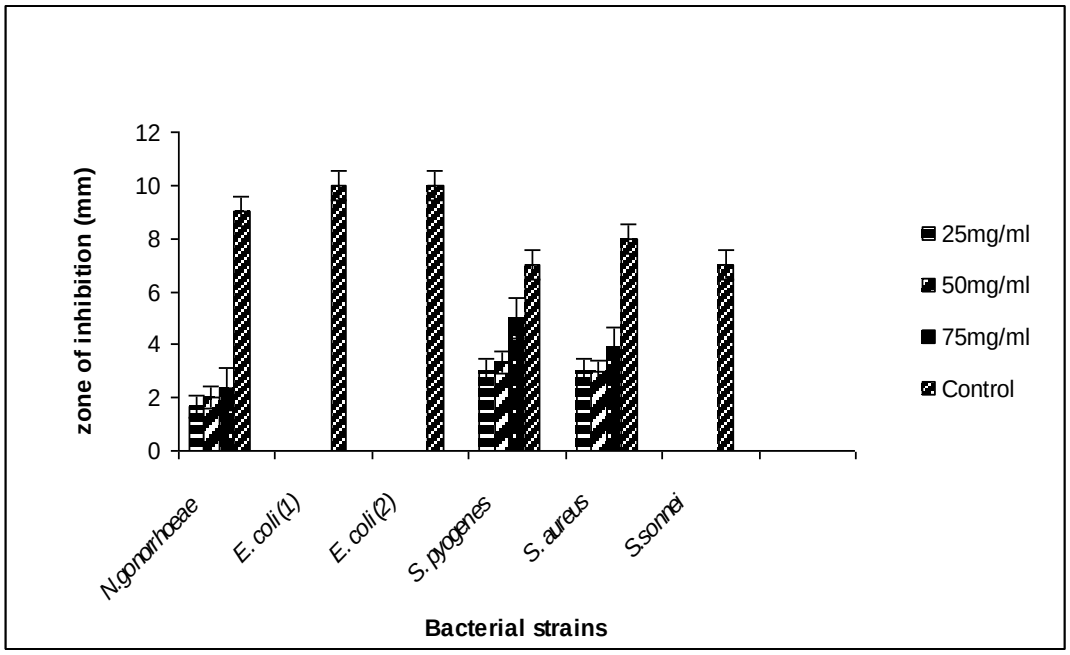

Fig. 6: Zone of inhibition of different bacterial strains after incubation of 48 hours at different concentrations of aqueous extract of *Manilkara zapota.*

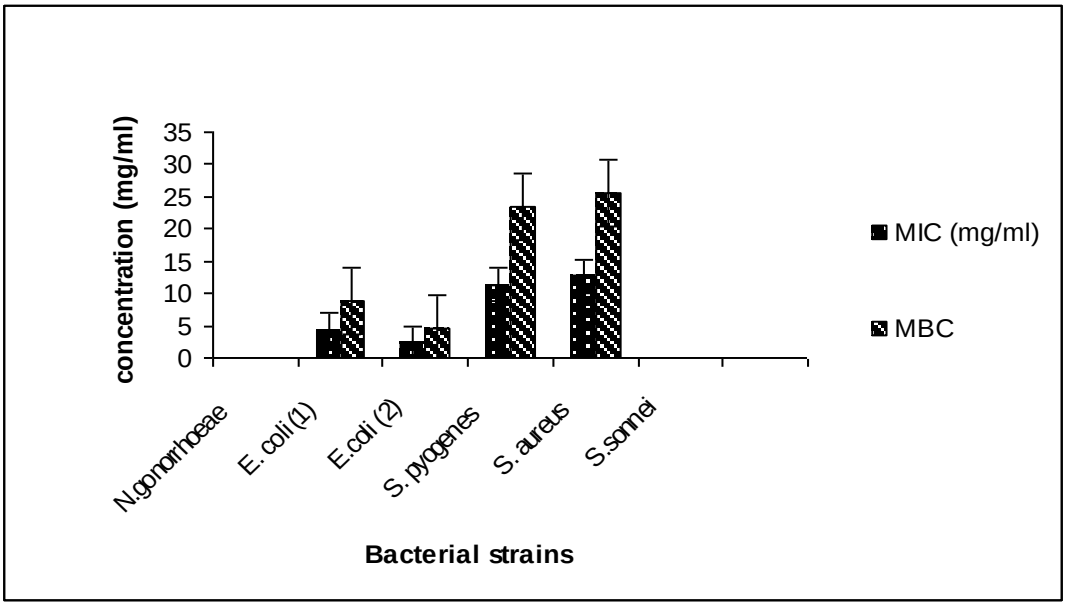

Fig. 7: Minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of methanolic extract of *M*anilkara *zapota.*

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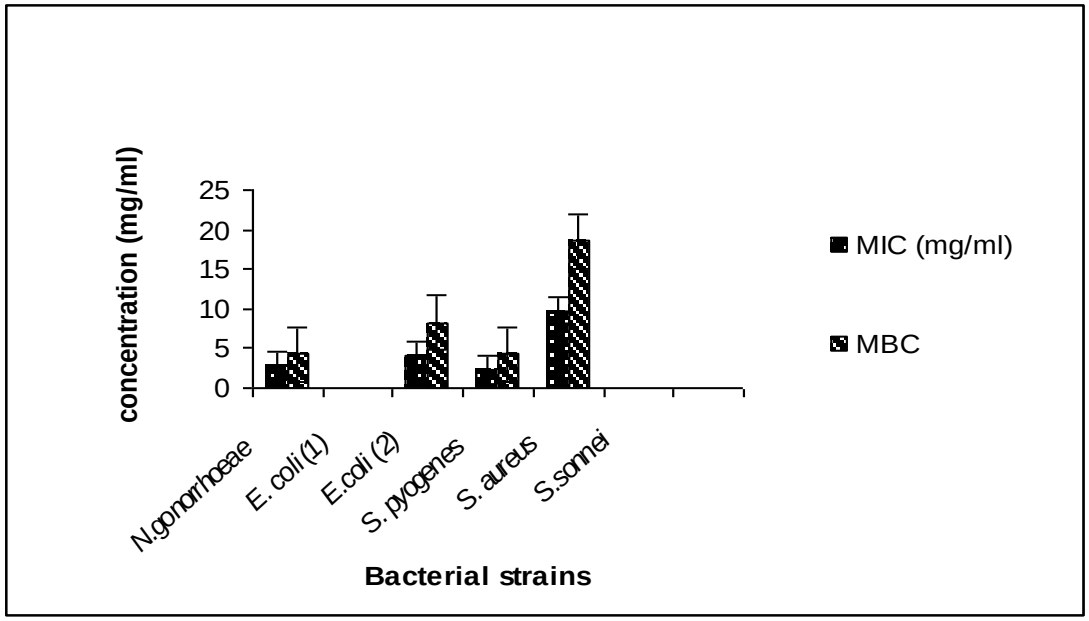

Fig. 8: Minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of ethanolic extract of *M*anilkara *zapota*.

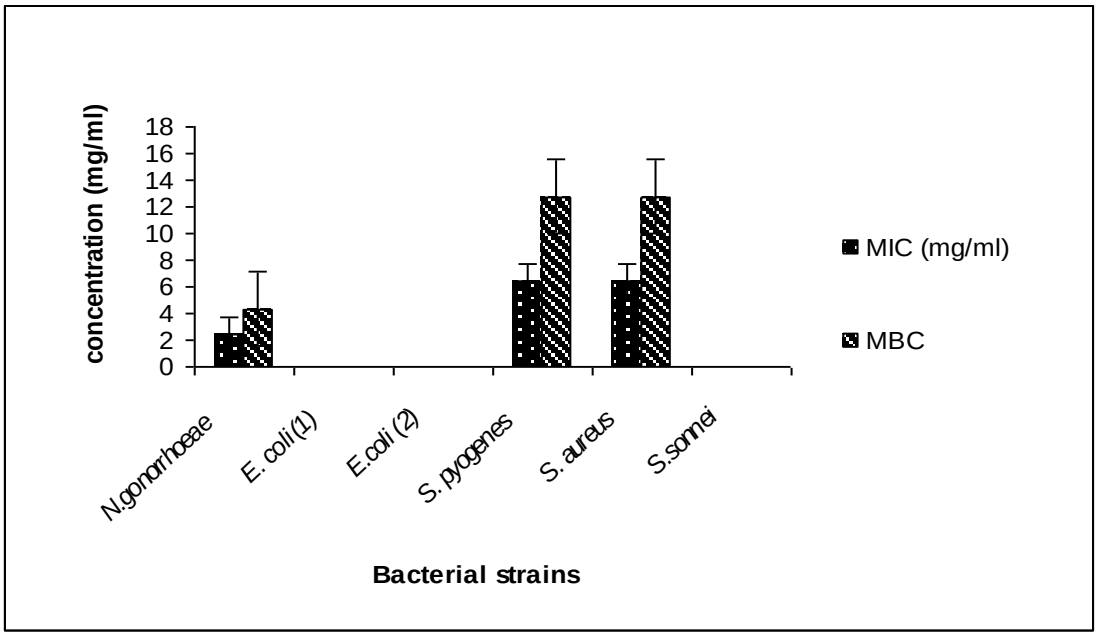

Fig. 9: Minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC) of aqueous extract of *M*anilkara *zapota*.

DISCUSSION

The antibacterial and antifungal potential of *Manilkara zapota* in methanolic, ethanolic and aqueous extracts was evaluated against six Gram-positive and Gram-negative bacteria and three fungal strains at different concentrations (25mg/ml, 50mg/ml, 75mg/ml) and at different incubation periods (24, 48). Antimicrobial activities were recorded in terms of zone of inhibition (mm) and compared to positive (Gentamicine) and negative controls Dimethyl sulphoxide (DMSO). In *M. zapota*, methanolic and ethanolic extracts showed maximum inhibition for *S. aureus*. Ethanolic and aqueous extract showed no inhibition against *S. sonnei*.

S. Sonnei is Gram negative bacteria. It has an outer membrane which acts as permeability barrier and restricts the entry of antimicrobial compounds and *S. aureus* is Gram positive bacteria. The single membrane of Gram positive bacteria is considerably more accessible to permeation by plant extracts (Parekh and Sandri, 2007; Chandra, 2010).

Our findings are supported by Priya *et al*. (2014), who studied the antioxidant and antibacterial activities of *M. zapota*. Methanolic and aqueous extracts of plants were used. Both extracts exhibited significant antibacterial activity against bacterial strains with zones of inhibition ranging from 26 mm to 29 mm. The zone of inhibition of methanol extract was the highest for *B. subtilis* (29 mm), followed by *S. aureus* (28.5) and in aqueous extract *S. aureus* showed significant growth inhibition (28.5). Osman *et al*. (2011) also determined that ethyl acetate extract of leaves showed mild activity against only *Staphylococcus aureus, Escherichia coli* and *Salmonella typhi* with inhibition zones in the range of 06-09 mm. In methanolic and ethanolic extract of *M. zapota*, alkaloids, saponins, flavonoids, glycosides, sugar and phenols were present and in aqueous extract of *M. zapota*, alkaloids, saponins, tannins, flavonoids and phenols were present. Priya *et al*. (2014) also confirmed phenols, tannins, alkaloids, flavonoids and saponins in the *M. zapota* extracts.

CONCLUSION

Our findings provide useful information for the potential use of the *Manilkara zapota* in further studies for discovering novel aspects about their antibacterial potential. The study is significant enough to encourage other researchers in the field to evaluate the striking potential of this plant in the combat of common disease causing pathogens. It also throws light on the importance of these plants for laymen and local people. Large scale growth of this plant makes

harnessing their antibacterial capabilities easy and low cost which is economical and can hugely aid local people who do not have access to high tech equipment for warding off pathogens that damage their crops.

REFERENCES

Abiramasundari, P., V. Priya, G.P. Jeyanthi and S. G. Devi. 2011. Evaluation of the Antibacterial activity of *Cocculus hirsutus*. *Hygeia. J. Dru. Medi*., 3(2): 26-31.

Ahmad, M., M.N. Mehjabeen, N. Jahan, M. Ahmad, Q.M. Mahmood and S.U. Jan. 2012. Spasmolytic effects of Scrophularia nodosa extract on isolated rabbit intestine. *Pak. J. Pharm. Sci*., 25(1): 267-275.

Alipour, M., and O. Khanmohammadi. 2011. Antibacterial activity of plant extracts against oral and skin pathogens. *Afri. J. Micro. Res*., 5(19): 2909-2911.

Anisimov, A.P., K.K. Amoako. 2006. Treatment of plague: promising alternatives to antibiotics. *Jour. Med. Microbiol*., 55(11): 1461-75.

Anonymous. 2004. The global epidemiology of infectious diseases. Global burden of diseases and injury series. Vol. I. available online at whqlibdoc.who.int/publications/ 2004/92303.pdf.

Bhalodia, N. R., P.B. Nariya and V. J. Shukla. 2011. Antibacterial and Antifungal activity from lower Extracts of Cassia fistula L: An Ethnomedicinal Plant. *Int. J. Pharm. Tech. Res*., 3(1): 160-168.

Bharath, K., R., S. Asha and B. S. Babu. 2014. A note on phytodiversity and phytochemistry of important plant species of Vignan University Campus, Vadlamudi, Andhra Pradesh. *Int. J. Pharma. Bio Sci*., 5(1): 373 -386.

Bibi, Y., S. Nisa, F.M. Chaudhary, M. Zia. 2011. Antibacterial activity of some selected medicinal.plants of Pakistan. *BMC Complement Altern Med*., 11(52)**:** 1-7.

Biren, S., M. Dikshit, D. Rajvi. 2012. Screening for Diuretic Potential of *Manilkara zapota* Seeds: Diuretic activity of *Manilkara zapota* seeds. Lambert Acadamic Publishing.

Cos, P., A. J. Vlietinck, D.V. Berghe, L. Maes. 2006. Anti-infective potential of natural products: how to develop a stronger in vitro proof-of concept. *J. Ethnopharmacol.*, 106:290–302.

Ergene, A., P. Guler, S. Tan, S. Mirici, E. Hamzaoglu and A. Duran. 2006. Antibacterial and antifungal activity of Heracleum sphondylium subsp. artvinense. *Afri. J. Biotech.*, 5: 1087-1089.

Evans, H.C. 1996. *Parthenium hysterophorus*: a review of its weed status and the possibilities for biological control. Biocontrol/News and Information. 18: 89-98.

Gullece, M., A. Aslan, M. Sokmen, F. Sahin, A. Adiguzel, G. Agar, A. Sokmen. 2006. Screening the antioxidant and antimicrobial properties of the lichens *Parmelia saxatilis*, *Platismatia glauca*, *Ramalina pollinaria*, *Ramalina polymorpha* and *Umbilicaria nylanderian*. *Phytomedi*, 13: 515–521.

Gupta, O. P., R. L. Kainsa, K. S. Dhawan and Chauhan. 1981. Post-harvest studies in fruits comparison of sugar for the preparation of candies. *Haryana Agric. Univ. Res. J*., 11(3): 389-392.

Hussain, A., 1. Wahab, I. Zarin and M. D. Sarfaraj Hussain. 2010. Antibacterial Activity of the Leaves of *Coccinia indica* (W. and A) India. *Adv. Biol. Res*., 4 (5): 241-248.

Islam, M.R., M.S. Parvin, M.R. Banu, N. Jahan, N. Das, M.E. Islam. 2013. Antibacterial and phytochemical screening of ethanol extracts of *Manilkara zapota* leaves and bark. *Int. J. Pharma Sci*., 3(6): 394-397.

Kiran, B. and K.A. Raveesha. 2006. Antifungal activity of seed extract of *Psoralea corylifolia L*. *Plant Dis. Res.*, 20: 213-215.

Kothari, V. and S. Seshadri. 2010. In vitro antibacterial activity in seed extracts of *Manilkara zapota*, *Anona squamosa*, and *Tamarindus indica. Biol. Res*., 43(2): 165-168.

Mahmoodally, M.F., A. Gurib-Fakim and A.H. Subratt. 2010. Screening for alternative antibiotics: an investigation into the antimicrobial activities of medicinal food plants of Mauritius. *Jour. Food. Sci.*, 75 (3): 173-177.

Maregesi, S.M., L. Pieters, O.D. Ngassapa, S. Apers, R. Vingerhoets, P. Cos, D.A. Berghe and A. Vlietinck. 2008. Screening of some Tanzanian medicinal plants from Bunda district for antibacterial, antifungal and antiviral activities. *J. Ethnopharmacol.,* 119:58–66.

Nair, R., and S. V. Chanda. 2007. Antibacterial Activities of Some Medicinal Plants of the Western Region of India. *Turk. J. Biol*., 31: 231-236.

Nananda and Connor. 1987. Estimating worldwide antibiotic usage: Report of the taskforce, *Clin. Infect. Dis*., 9(3): 232-234.

Okigbo, R.N. and U.O. Ogbonnaya. 2006. Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post-harvest yam (Dioscorea spp.) rot. *Afri. J. Biotech.*, 5: 727-731.

Osman, M.A., M.A. Aziz, M.R. Habib and M.R. Karim. 2011. Antimicrobial investigation on *Manilkara zapota* (L.) P. Royen. *Int. J. Drug. Dev. Res*., 3(1): 185-190.

Pandya, K., B. Solanki, B. Shah, D. Parekh, H. Soni, K. Maniar, N. Gurav and P. Patel. 2011. Phyto-chemical Screening & Evaluation of Antibacterial Activity of Polyherbal Formulation. *Indo-Global J. Pharm. Sci*., 1(3): 206-218.

Parekh, J., and S. Chanda. 2007. *In vitro* antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* Kurz. Flower (Lythraceae). *Braz. J. Microbiol*., 38:204-207.

Paterson, D. L. 2008. Impact of antibiotic resistance in gram-negative bacilli on empirical and definitive antibiotic therapy. *Clin. Infect Dis*., 15(47):14–20.

Roopashree, T.S., R. Dang, S. Rani, R.H., C. Narendra. 2008. Antibacterial activity of antipsoriatic herbs: *Cassia tora, Momordica charantia* and *Calendula officinalis. Inter. J. App. Res. Nat. Pro*., 1(3): 20-28.

Satish, S., K.A. Raveesha and G.R. Janardhana. 1999. Antibacterial activity of plant extracts on phytopathogenic Xanthomonas campestris pathovars. *Lett. Appl. Microbiol*., 28: 145–147.

Savoia, D. 2012. Plant derived antimicrobial compounds: alternatives to antibiotics. *Future Microbiol.,* 7(8): 979-999.

Shariff, N., M.S.Sudarshana, S. Umesha and P. Hariprasad. 2006. Antimicrobial activity of *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracts. Afri. *J. Biotech.,* 5: 946-950.

Vashist, H., A. Jindal. 2012. Antimicrobial Activities of Medicinal Plants–Review. *Int. J. Res. Pharma Biomed. Sci*., 3:222-230.

Vijay, K. and S. Sriram. 2010. *In vitro* antibacterial activity in seed extracts of *Manilkara zapota*, *Anona squamosa*, and *Tamarindus indica*. *Biol. Res.,* 43*:* 165-168.

Yala, D., A.S. Merad, D. Mohamedi and K.M. Ouar. 2001. Classification et mode d'action des antibiotiques. *Medecine du Maghreb*., (91):5-12.

Yashvanthkumar, D., R. V. Mayankagarval, P. Sruthy and V. Krishna. 2012. *Manilkara zapota* seed embryo extract a potent anthelminthic agent. *Asian. J. Pharma. Clin. Res*., 5(3): 159-161.

Zhang, R., K. Eggleston, V. Rotimi and R.J. Zeckhauser. 2006. Antibiotic resistance as a global threat: evidence from China, Kuwait and the United States. *Global Health*. 7; 2:6.