Antibacterial activity of bark extracts of *Moringa oleifera* Lam. against some selected bacteria

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Abstract: The methanol, chloroform, ethyl acetate and aqueous bark extracts of *Moringa oleifera* were evaluated for their antibacterial activity against four bacteria viz. *Staphylococcus aureus*, *Citrobacter freundii*, *Bacillus megaterium and Pseudomonas fluorescens* using erythromycin as positive control. The activity was analyzed using paper disc diffusion method at different concentration of the extract. The study revealed that all the bark extracts irrespective of their types, in different concentrations inhibited growth of the test pathogens to varying degrees. Ethyl acetate extract showed maximum activity against all the bacterial strains followed in descending order by chloroform, methanol and aqueous extracts. The activity decreased with decrease in concentration of the extract. *Staphylococcus aureus* was found to be the most sensitive test organism to different extracts of *Moringa oleifera.* Looking to these results it may be concluded that *M. oleifera* may be a potential source for the treatment of different infections caused by the resistant microbes.

Keywords: Antibacterial, disc diffusion, erythromycin, *Moringa oleifera*.

INTRODUCTION

Interest in plants with antimicrobial properties has been revived as a result of antimicrobial resistance. The microorganisms have developed resistance to many antibiotics because of indiscriminate use of antimicrobial drugs that create a big problem in the treatment of infectious diseases (Davis, 1994). With the increase in resistance of many microorganisms to the currently used antimicrobials and the high cost of production of synthetic compounds; in addition to many side effects; there is a need to look for the alternatives. Plants have provided a good source of anti infective agents; emetine, quinine, berberine, tannins, terpenoids, alkaloids and flavonoids remain highly effective instruments in the fight against microbial infections (Marjorie, 1999).

The frequency of life threatening infections caused by pathogenic microorganisms has increased worldwide and is becoming an important cause of morbidity and mortality in immune-compromised patients in developing countries (Al-Bari *et al.,* 2006). In the last few decades there has been an exponential growth in the field of herbal medicines. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects (Brahmachari, 2001). It is therefore, very necessary that the search for newer antibiotic sources should be a continuous process. Plants are the cheapest and safe alternative source of antimicrobials (Pretorus and Watt, 2001; Sharif and Banik, 2006 and Doughari *et al.*, 2007).

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One of such plants of medicinal value is *Moringa oleifera*, belongs to the family Moringaceae, commonly known as Sahajan in Hindi. The different parts of this plant viz. leaves, stem bark, root bark, flowers, fruits and seeds are used in the indigenous systems of medicine for the treatment of variety of human ailments (Chopra *et al.*, 1956; Nadkarni and Nadkarni, 1976).

Despite the array of uses to which parts of *Moringa oleifera* tree are put to, scanty literature is available on the uses of *Moringa oleifera* bark as antimicrobial. However, a very important step in the screening of a plant material for antimicrobial activity is to evaluate its antibacterial activity against pathogenic microorganisms. The determination of a plant's antibacterial profile against microorganisms may promote the plant to further tests in order to validate its popular use.

MATERIALS AND METHODS

Collection of plant material

The bark of *M. oleifera* of varying age was collected from different areas of Agra region. Collected material was shade dried, made to coarse powder and then packed in polythene bags for further analysis.

Extraction of active principles

Soxhlet extraction method following (Okeke *et al.,* 2001) was used for the extraction of *M. oleifera* bark. The weighed amount of *M. oleifera* bark powder was packed in extraction thimble and placed in an extraction chamber which was suspended above the flask containing the solvent and below a condenser. The flask was heated and

the solvent evaporated and moved into the condenser where it was converted into a liquid that trickled into the extraction chamber containing the plant material. The extraction chamber was designed so that when the solvent surrounding the sample exceeded a certain level it overflowed and trickled back down into the boiling flask. At the end of the extraction process, the flask containing the extract was removed and solvent was evaporated by using rotary evaporator. The crude extract was stored in refrigerator for antimicrobial analysis.

Test organisms

The pure cultures of test bacterial strains used in the study were *Staphylococcus aureus* (MTCC-740), *Citrobacter freundii* (MTCC-1658), *Bacillus megaterium (*MTCC-428*)* and *Pseudomonas fluorescens* (MTCC-103). The strains were obtained from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh. The strains were inoculated in the freshly prepared nutrient broth liquid medium and incubated at 37^{o}C. Aseptically some colonies from the pure culture was mixed (emulsify) in nutrient broth (7µl/ml broth) and incubated at 37^{o}C overnight prior to antibacterial sensitivity. At the time of screening agar plates were inoculated with the bacterial cell culture (10^8 CFU/ml) by using 0.5 McFarland turbidity standards using sterile culture moistened cotton swab.

Antibacterial activity assay

In vitro antibacterial activity of selected plant extracts were tested by disc diffusion method (Kohner *et al.*, 1994). For susceptibility testing, crude extract was made into a suspension using Dimethyl Sulfoxide (DMSO). The concentration of the material was made 200mg/ml and further concentrations were prepared by serial dilution method. Sterile discs having a diameter of 6 mm were impregnated with 25/µl of each serial dilution of extracts and dried in an incubator to remove the solvent. The plates were inoculated with the bacterial cell culture (10^8 CFU/ml) by using 0.5 McFarland turbidity standards. Sterile discs loaded with extracts were placed on inoculated surface of agar plate with the help of sterile forceps. These plates were incubated for 24 hours at 37ºC. The diameter of the zones of inhibition around each of the disc was taken as measure of the antibacterial activity. Each experiment was carried out in triplicate and mean diame-ter of the inhibition zone was measured in millimeter.

STATISTICAL ANALYSIS

Results are expressed as Mean ± S.D.

RESULTS

The bark extracts of *Moringa oleifera* were analyzed for their antibacterial activity against *S. aureus, C. freundii, B. megaterium* and *P. flouresence*. The present study revealed that antibacterial efficacy of *M. oleifera* is age dependent. The aged plants showed potent antibacterial activity than the young ones.

The antibacterial activity of ethyl acetate extract is clearly shown in Figs. 1a-1d. It was observed that the extract was more active against *S. aureus, B. megaterium* and *P. flouresence*, which showed inhibitory effect up to dilution of 6.25mg/ml while it was comparatively less active against *C. freundii* showing zone of inhibition up to dilution of 12.5mg/ml (table 1 and figs. 1 and 1a-d).

Figs. 2a-2d reveals the antibacterial activity of methanol extract of *Moringa oleifera* bark. The extract was found to be most active against *C. freundii* and *P. flouresence*, showed zone of inhibition up to dilution of 3.125mg/ml while it was observed that methanol extract of bark was slightly less active against *S. aureus* and *B. megaterium* having zone of inhibition up to dilution of 6.25mg/ml (table 2 and fig. 2).

The antibacterial activity of aqueous extract of *Moringa oleifera* bark is shown in figs. 3a-3d. *P. flouresence* was the most sensitive test organism to the aqueous extract of *M. oleifera* bark and the zone of inhibition was observed up to dilution of 3.125mg/ml while in case of *B. megaterium* and *C. freundii* the inhibition was found up to dilution of 6.25mg/ml (table 3 and fig. 3).

It is clear from figs. 4a-4d that chloroform extract of *M. oleifera* bark had the activity against the test pathogens. Maximum antibacterial activity was found against *P. flouresence*, showing zone of inhibition up to dilution of 3.125mg/ml and moderate antibacterial activity against *S. aureus* having inhibition up to 6.25mg/ml and the minimum antibacterial activity against *B. megaterium* and *C. freundii* with inhibition up to 12.5mg/ml (table 4 and fig. 4).

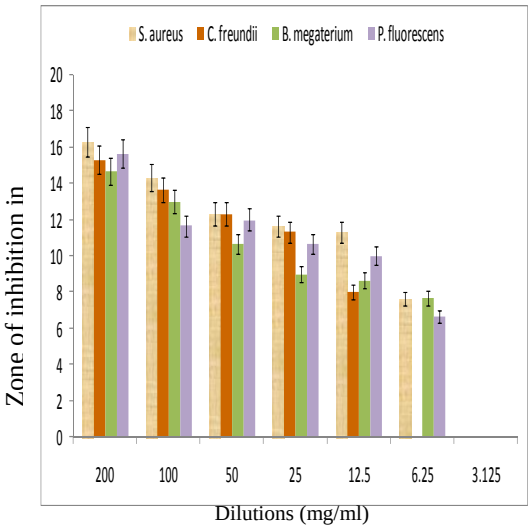


Fig. 1: Graphical representation of antibacterial activity of *M. Oleifera* bark ethyl acetate extract.

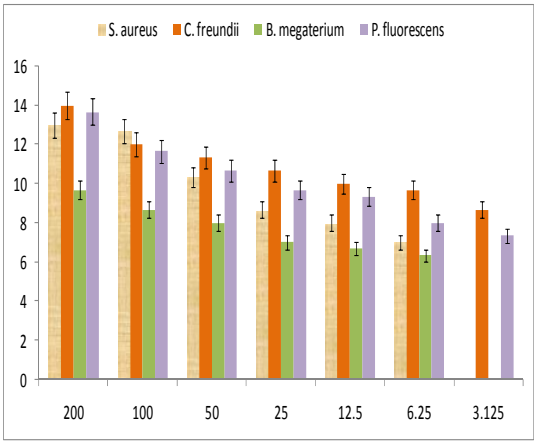


Fig. 2: Graphical representation of antibacterial activity of *M. oleifera* bark methanol extract.

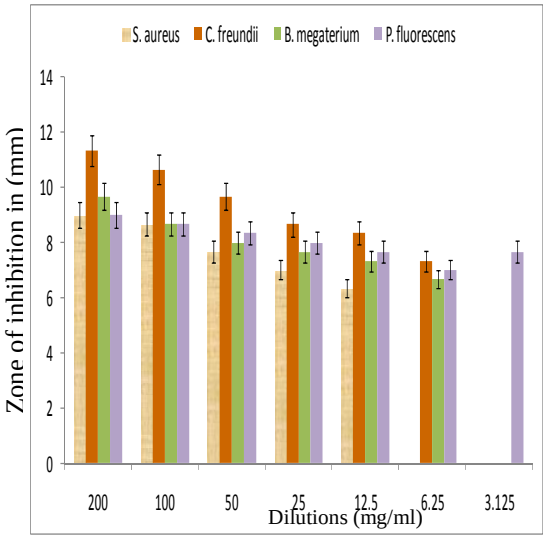


Fig. 3: Graphical representation of antibacterial activity of *M. oleifera* bark aqueous extract

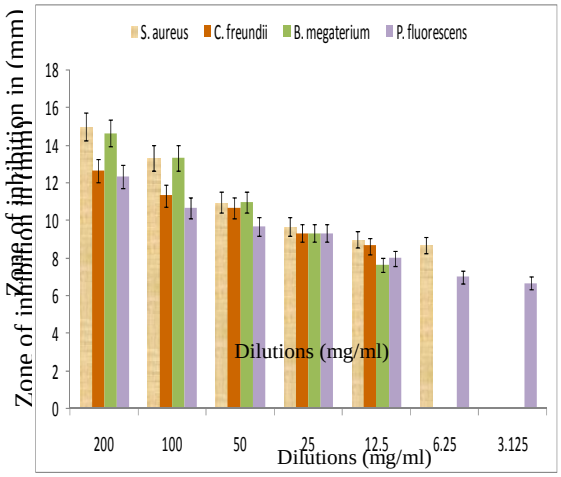
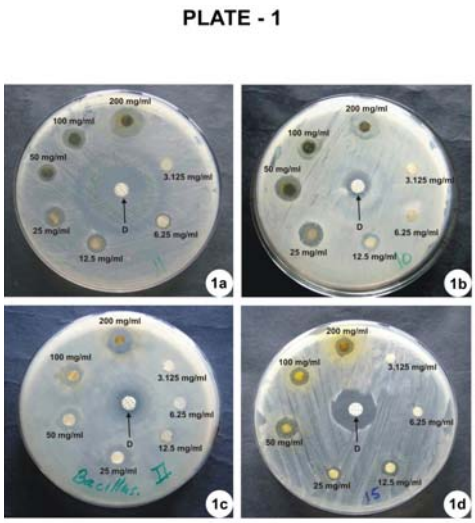
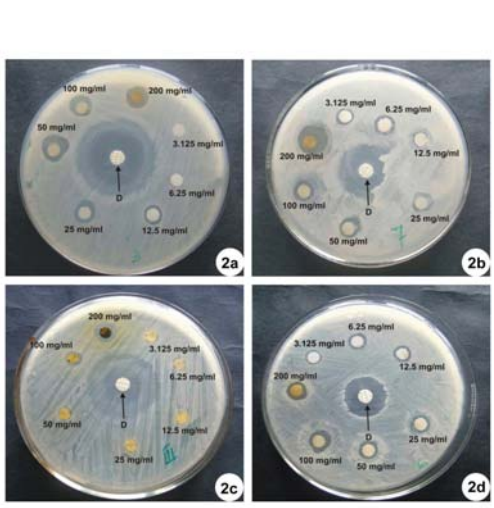


Fig. 4: Graphical representation of antibacterial activity of *M. oleifera* bark chloroform extract.



D = drug as control



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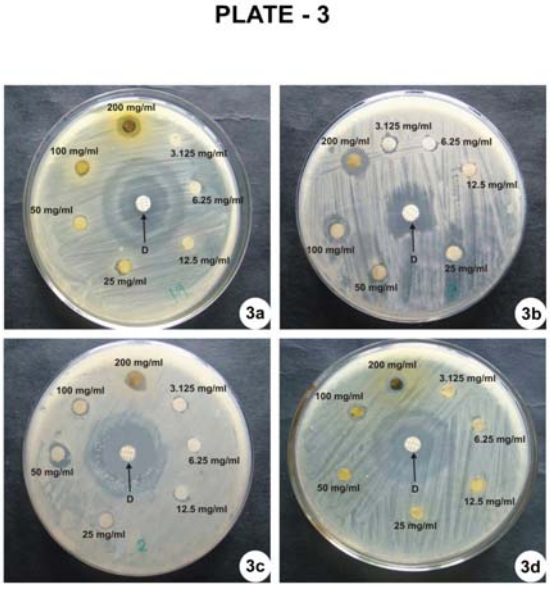
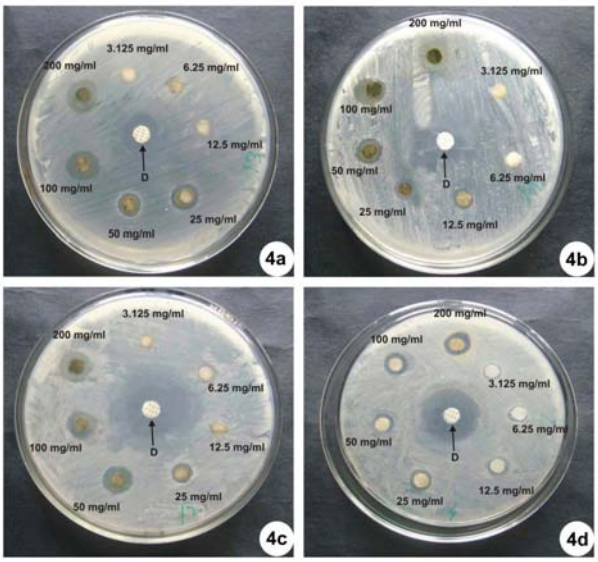


D = drug as control

PLATE - 4



D = drug as control

DISCUSSION

Medicinal plants remain an important source of new drugs, new drug leads and New Chemical Entities (NCEs). It has been reported that many medicinal plants are rich in varieties of secondary metabolites such as alkaloids, flavonoids, tannins and terpenoids. These secondary plant metabolites exert a wide range of biological activities on physiological systems. In the present study, methanol, chloroform, ethyl acetate and aqueous bark extracts of *Moringa oleifera* were evaluated for their antibacterial activity against four bacteria viz. *Staphylococcus aureus*, *Citrobacter freundii*, *Bacillus megaterium and Pseudomonas fluorescens.* Antibacterial properties of bark of *M. oleifera* as shown in present study corroborate the earlier claims by Dewangan *et al* (2010) who reported that ethyl acetate has maximum antibacterial activity against *S. aureus.*

Methanol bark extract showed promising antibacterial activity to most of the test pathogens. Similar result was observed in the study of Rao *et al* (2011) who investigated antibacterial activity of methanolic extract of *M. oleifera* by using well diffusion technique and reported that the most significant activity of this plant was seen against *S. aureus.* While working on same plant species Devi *et al* (2011) investigated the antibacterial activity of methanolic extract of bark by agar well diffusion method against *Bacillus spp.* and *S. aureus.* The results are also in agreement with Ahmad *et al* (2012) who reported methanolic leaf extract of *C. australis* had the highest activity against *S. aureus* at 200mg/ml concentration with 10.5mm zone of inhibition. The methanol extract of bark have shown strong antibacterial activity against the test organisms. McCutcheon *et al* (1992) reported that most of the plant extracts shows activity against gram-positive than gram-negative bacteria. But in our present study, methanol extract possess better inhibitory activity against gram-negative than gram-positive bacteria. This revealed the medicinal potential against gram-negative bacteria.

The aqueous extract also moderately inhibited the growth of test pathogens. Our results are in agreement with Caceres *et al* (1991) who reported antimicrobial activities of *Moringa oleifera*, bark *in vitro* against bacteria, yeast, dermatophytes and helminths by disk-diffusion method. The aqueous extract from the bark inhibit the growth of *Pseudomonas spp* and *S. aureus.* The aqueous extract of *Moringa oleifera* bark has shown strong antibacterial activity against the test organisms.

Table 1: Antibacterial activity of *M. oleifera* bark ethyl acetate extract against different bacteria

Pathogens	Zone of inhibition in (mm)							
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25 mg/ml	3.125 mg/ml	Control (30 mcg)
S. aureus	16.33±2.08	14.33±3.21	12.33±2.51	11.67±2.08	11.33±1.52	7.66±0.57	-	20
C. freundii	15.33±1.53	13.67±1.08	12.33±1.51	11.33±0.58	8.00±1.00	-	-	13
B. megaterium	14.67±2.88	13.00±1.73	10.67±1.15	9.00±.52	8.66±0.58	7.67±0.57	-	14
P. fluorescence	15.67± .15	11.67±1.52	12.00±1.25	10.66±1.15	10.00±1.00	6.67±0.58	-	18

Table 2: Antibacterial activity of *M. oleifera* methanol bark extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	Control (30 mcg)
S. aureus	13.00±1.52	12.67±1.53	10.33±0.57	8.66±1.53	8.00±1.00	7.00±0.58	-	20
C. freundii	14.00±2.05	12.00±2.60	11.33±1.73	10.66±1.15	10.00±1.00	9.66±0.58	8.66±0.57	18
B. megaterium	9.66±1.52	8.67±0.58	8.00±1.00	7.00±2.00	6.67±0.58	6.33±0.57	-	18
P. fluorescens	13.67±1.15	11.66±1.53	10.66±1.52	9.67±1.15	9.33±0.58	8.00±1.00	7.33±0.57	16

Table 3: Antibacterial activity of *M. oleifera* aqueous bark extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	Control (30mcg)
S. aureus	9.00±1.15	8.67±0.58	7.67±1.00	7.00±0.57	6.33±0.58	-	-	17
C. freundii	11.33±0.58	10.66±1.15	9.66±0.58	8.66±1.00	8.33±1.15	7.33±0.57	-	16
B. megaterium	9.66±0.58	8.67±1.15	8.00±1.00	7.66±1.15	7.33±0.57	6.67±0.58	-	18
P. fluorescens	9.00±1.00	8.67±0.58	8.33±0.57	8.00±1.00	7.67±0.58	7.00±1.00	7.66±0.58	13

Table 4: Antibacterial activity of *M. oleifera* chloroform bark extract against different test microorganisms.

Pathogens	Zone of inhibition in (mm)							
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	Control (30mcg)
S. aureus	15.00±1.00	13.33±1.52	11.00±1.73	9.67±0.58	9.00±1.00	8.67±0.58	-	13
C. freundii	12.67±1.53	11.33±1.52	10.67±0.57	9.33±1.53	8.66±0.57	-	-	14
B. megaterium	14.67±2.08	13.33±1.73	11.00±1.00	9.33±1.16	7.66±0.58	-	-	16
P. fluorescens	12.33±1.53	10.67±0.58	9.67±0.58	9.33±1.15	8.00±1.00	7.00±0.57	6.67±0.58	17

±: standard deviation, Control: Erythromycin

The chloroform bark extract showed best activity against *Pseudomonas flouresence.* Dewangan *et al* (2010) reported that *S. aureus* and *Pseudomonas spp.* showed variable sensitivity to different extracts of *M. oleifera*. The sensitivity of the above bacteria to chloroform extract was in between the ethyl acetate and aqueous extracts.

CONCLUSION

Based on these results, it could be conclude that ethyl acetate was the best extractive solvent for antibacterial activity of *Moringa oleifera* against all the tested organisms. The activity decreased with the decrease in the concentration of the extract. The varying degrees of sensitivity of the bacterial test organisms may be due to the intrinsic tolerance of microorganisms. However, it is important to determine the specific compounds responsible for the antimicrobial activity as well as to establish the mechanism of action of the extract to come to a definite conclusion.

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REFERENCES

Ahmad S, Sharma R, Mahajan S and Gupta A (2012). Antibacterial activity of *Celtis australis* by *in vitro* study. *Int. J. Pharm. Pharm. Sci.*, **4**(2): 629-631.

Al-Bari MA, Sayeed MA, Rahman MS and Mossadik MA (2006). Characterization and antimicrobial activities of a phenolic acid derivative produced by *Streptomyces bangladeshiensis*, a novel species collected in Bangladesh. *Res. J. Med. Med. Sci.*, **1**: 77-81.

Bramachari UN (2001). The role of science in recent progress of medicine. *Curr. Sci.*, **81**(1): 15-16.

Caceres A, Cabrera O, Morales O, Mollinedo P and Mendia P (1991). Pharmacological properties of *Moringa oleifera*. *J. Ethnopharmacol.*, **33**(3): 213-216.

Chopra RN, Nayar SL and Chopra IC (1956). *Glossary of Indian Medicinal Plants*, 1st Edition, CSIR, New Delhi.

Davis J (1994). Inactivation of antibiotics and the dissemination of resistance genes. *Science*. **264**: 375-382.

Devi GS, Priya V, Abiramasundari P and Jeyanthi PG (2011). Antibacterial activity of the leaves, bark, seed and flesh of *Moringa oleifera*. *Int. J. Phar. Sci. and Res.*, **2**(8): 2045-2049.

Dewangan G, Koley KM, Vadlamudi VP, Mishra A, Poddar A and Hirpurkar SD (2010). Antibacterial activity of *Moringa oleifera* (drumstick*)* root bark. *J. Chem. Pharm. Res*., **2**(6): 424-428.

Doughari JH, El-mahmood AM and Manzara S (2007). Studies on the antibacterial activity of root extracts of *Carica papaya* L. *Afri. J. Microbiol. Res.,* **1**(3): 037-041.

Kohner PC, Rosenblatt JE and Cockerill FR (1994). Comparison of agar dilution, broth dilution and disk diffusion testing of Ampicillin against *Haemophilus* spp. by using in house and commercially prepared media. *J. Clin. Microbiol.,* **32**: 1594 -96.

Marjorie MC (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev.*, **12**: 564-582.

McCutcheon AR, Ellis SM, Hancock REW, Towers GHN (1992). Antibiotic screening of medicinal plants of the British Columbian native peoples. *J. Ethnopharmacol*., **37**: 213-223.

Nadkarni KM and Nadkarni AK (1976). The Indian Materia Medica, 4th Edition, Popular Prakashan Pvt. Ltd., Bombay, India, pp.810-816.

Okeke MI, Iroegbu CU, Eze EN, Okoli AS and Esimone CO (2001). Evaluation of extracts of the root of *Londolphia owerrience* for antibacterial activity. *J. Ethanopharmacol.*, **78**: 119-127.

Pretorius CJ and Watt E (2001). Purification and identification of active components of *Carpobrotus edulis L. J. Ethnopharmarcol.*, **76**: 87-91.

Rao PK, Rao DB, Ravi CK, Nadh MR, Madhavi Y and Rao TR (2011). *In vitro* antibacterial activity of *Moringa oleifera* against dental plaque bacteria. *J. Pharmacol. Res*., **4**: 3.

Sharif MDM and Banik GR (2006). Status and utilization of medicinal plants in Rangamati of Bangladesh. *Res. J. Agric. Biol. Sci.*, **2**(6): 268-273.