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EVALUATION OF PHYTOCHEMICALS AND ANTIBACTERIAL ACTIVITY OF *MORINGA OLEIFERA* AVAILABLE IN THE MARKET OF RAIPUR, CHHATTISGARH

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ABSTRACT

The rate at which bacteria are resistant to the commercial antibiotics meant for their prevention and cure is of great concern. In view of this, many research groups are now engaged in medicinal plant research because natural products of higher plants may give a new source of antimicrobial agent. The antibacterial activity of *Moringa oleifera* (Lam.) Leaves extract belonging to the family Moringaceae, was determined using agar well diffusion method against some selected bacteria. Mueller Hinton Agar (MHA) (Becton Dicknson M. D USA), media was prepared according to the manufacturer's instruction. The present Study is the continuation of a program aimed at investigation of antimicrobial properties of *Moringa oleifera* leaf extract. The antibacterial activity was evaluated according to the disk diffusion method by using Gram positive; *B. subtilis*, *S. aureus* and *Enterococcus* and Gram negative; *E. coli*, *Acetobacter*, *Citrobacter*, bacteria. Concentrations of 25, 50, 75 and 100 mg/ml prepared from the dry leaves powder were used for antibacterial analysis using agar well diffusion methods. The methanol extracts of the plant leaves show an inhibitory effect on the growth of the tested bacteria. This Study shows that methenolic leaves extracts of *Moringa oleifera* Linn inhibit the growth of microorganism's dose dependently. Phytochemical analyses of the leaf in solvents of varying polarity with methanol extract were also carried out. The phytochemical screening indicated the presence of flavonoids, tannins, steroid, alkaloid, saponins etc., in the extracts. The research showed that the higher reducing power of the methanolic extract could be due to the better solubility of the antioxidant components in water whereas the predominant antibacterial activity in organic solvent extracts, indicates that the active components responsible for the bactericidal activity are more soluble in organic solvents. These studies provide an evidence to support traditional medicinal uses of the plant.

KEYWORDS: Antibacterial Activity, *Moringa oleifera*, Disk Diffusion, Phytochemical Screening.

1. INTRODUCTION

Moringa oleifera is medium sized tree, about 10m height, found in the sub-Himalayan tract, (Trapti, *et. al*., 2009) which belongs to family Moringaceae, native to, Africa, Arabia, South Asia, South America, Himalaya region, India, Pakistan, the Pacific and Caribbean Islands. *Moringa oleifera* has been naturalized in many tropic and subtropics regions worldwide, the plant is referred to number of names such as horseradish tree, drumstick tree, oil tree, miracle tree, and "Mothers best friend" (Julia, 2008). *Moringa oleifera* is commonly known as "Drumstick". Moringa oleifera is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 to 12m in its height, open crown of drooping fragile branches, feathery foliage of trip innate leaves and thick corky, whitish bark (Roloff, *et. al*., 2009). The Moringa oleifera plant provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals (Pal, 1995). The leaves are used as a source of vitamins A and C. They are also good sources of vitamin B and are also sources of minerals (Talhaliani, 2000). Ethanolic extract of *Moringa oleifera* leaves (Faizi, *et al*., 1994) contain niazirin, niazirinin, niazininins A and B. Benzoic acid, gallicacid, beta benzaldehyde have been isolated from methanolic extract of Moringa oleifera leaves (Manguro, *et. al*., 2007). The leaf of this plant has diverse biological activities, including hypocholesterolemic, antidiabetic, hypertensive agent, (Mehta, *et. al*., 2003; Kar, *et. al*., 2003; Faiz, *et. al*., 1995; Guevara, *et. al*., 1991) and regulate thyroid hormone, (Tahiliani, *et. al*., 2004) central nervous system, digestive system, nutrition and metabolism. This plant is also reported to be hepato protective against anti tubercular drug such as ionized and rifampicin, (Pari, *et. al*., 2002; Fakurazi, *et. al*., 2008) and being studied for its anti-inflammatory, antimicrobial, diuretic, (Caceres, *et. al*., 1991; Caceres, *et. al*., 1992; Udupa, *et. al*., 1994) antibiotic, (Eilert, *et. al*., 1981) hypotensive and antimicrobial properties

(Palaniswamy, 2004). An immune enhancing polysaccharide (Mondal, *et. al*., 2004) and niaziminin, having structural requirement to inhibit tumor promoter induced Epstein Barr virus activation have been reported from the leaves (Murakami, 2008). The alcoholic extract of leaves of *Moringa oleifera* was reported to have analgesic activity (Nitin, *et. al*., 2008). Traditionally, the plant is used as antispasmodic, stimulant, expectorant and diuretic (Nadkarni, 2009). *Moringa oleifera* is used as a drug by many ayurvedic practitioners for the treatment of asthma and to evaluate the anthelmintic activity of methanolic extract of *Moringa oleifera* in adult ethopian earth worm's *Pheretima posithuma* at different doses (Iswar, *et. al*., 2010). According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs (Caceres, *et. al*., 1992). The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on human body. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Udupa, *et. al*., 1994). Despite this array of uses to which parts of Moringa tree are put to, scanty literature is available on the uses of *Moringa oleifera* plants as sanitizers or preservatives in foods. However, a very important step in the screening of a plant material for sanitizing/preservative activity was to evaluate its phytochemical and antibacterial activity.

2. MATERIALS AND METHODS

2.1 Plant material – *Moringa oleifera* leaves was collected from Local market, Raipur (Chhattisgarh), India.

2.2 Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

2.3 Preparation of extract - Dried powdered of *Moringa oleifera* leaf (10 g) were extracted by continuous mixing in 100 ml 50% methanol, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 0c temperature. The final extract was kept in air tied box.

2.4 Phytochemical screening of the extract

The portion of the dry extract was subjected to the Phytochemical screening using the method adopted by Trease, Evans and Harbourne. Phytochemical screening was performed to test for alkaloids, saponins, glycosid, proteins, phytosterols, flavonoids, triterpenoids, tannins fixed oil and sugar.

2.4.1 Test for Alkaloids: A small portion of the extract was stirred separately with 1 ml of dilute Hydrochloric acid and filtered. The filtrate was treated with Dragandroff's reagent. Appearance of organic precipitate shows the presence of alkaloids.

2.4.2 Test for Saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.4.3 Test for Glycosides: Small quantity of the extract was hydrolyzed with 5ml Hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to Fehling's test. To 2ml of Fehling's solution (1ml of Fehling's A and 1 ml of Fehling's B solution), 2ml of extract was added, mixed well and boiled. Appearance of yellow or red color precipitate indicates the presence of reducing sugars.

2.4.4 Test for Proteins: Small quantity of the extract was dissolved in 5 ml of water and subjected to Xantho protein test. To 3 ml of the extract, 1ml of concentrate Nitric acid was added. A white precipitate was obtained. The solution was heated for 1minute and cooled under tap water. It was made alkaline by excess of 40% NaOH. Appearance of orange precipitate indicates the presence of protein.

2.4.5 Test for Phytosterol: Salkowski test was done for the detection of phytosterols. In this test, 1 ml of concentrated Sulphuric acid was added to the 1g plant extract and allowed to stand for 5 minutes. After shaking, formation of golden yellow color in the lower layer indicates the presence of phytosterols.

2.4.6 Test for Flavonoids: The extract was treated with concentrated Sulphuric acid. Appearance of yellowish orange show the presence of anthocyanins, yellow to orange color show the presence of flavones, and orange to crimson show the presence of flavonones.

2.4.7 Test for Terpenoids (Salkowski test): 5 g of each extract was mixed in 2 ml of chloroform, and concentrated H_2S0_4 (3ml) was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.4.8 Test for Tannins: About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for browrish green or a blue-black colouration.

2.4.9 Reducing Sugars: To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath.

2.4.10 Volatile Oil: 2ml of Extract was shaken with 0.1ml dilute NaOH and a small quantity of dilute HCl.

2.5 Microorganisms: The tested microorganisms included the Gram positive bacteria; *Bacillus subtilis, Staphylococcus aureus, Enterococcus* and Gram negative bacteria; *Acetobacter, Citrobacter, Escherichia coli*. These bacteria's strains were procured from National Chemical Laboratory (NCL), Pune, India. The bacteria were grown in the nutrient broth at 37^0 C and maintained on nutrient agar slants at 4^0 C.

2.6 Culture media

The media used for the activation of the microorganisms was nutrient broth. The Nutrient agar media was used for the antimicrobial test. All the culture media were prepared and treated according to the manufacturer guidelines (Hi Media laboratories Ltd, Mumbai, India). Preparation of stock solution:

100%: 1gm drug + 1ml ddw (stock solution)
75%: 750µl stock solution + 250µl ddw
50%: 500µl stock solution + 500µl ddw
25%: 250µl stock solution + 750µl ddw

2.7 Antibacterial Assay: Antibacterial activity of *Moringa oleifera* leaf extract was determined by agar disk diffusion method (Nair, *et, al*., 2005) at four concentrations i.e., 100,75,50 and 25mg/ml. Muller Hinton agar was prepared according to the manufacturer's instructions and the plates were seeded with appropriate microorganisms (*Bacillus subtilis, Staphylococcus aureus, Enterococcus, Acetobacter, Citrobacter and Escherichia coli*). Discs of 6 mm diameter were prepared from Whatmann filter paper No. 24 and sterilized. The discs were then impregnated with the extracts and solvent DMSO. Antibiotics for Gram positive (NX – Norfloxacin, OF- Ofloxacin, E-Erythromycin, CFM- Cefixime) and Gram Negative (NX–Norfloxacin, OF- Ofloxacin, E-Erythromycin, CFM- Cefixime).Bacteria were used as standard. The plates were incubate at 37º C for 24 hrs and the zones of inhibition were measured with a measuring scale. Above experiment was carried out in triplicate for their confirmation.

2.8 Statistical Analysis

To evaluate associations between variables (antibiotic profiles), the data were analyzed statistically using mean + standard deviation and standard error.

3. RESULT

Phytochemicals screening

The present study reveals that *Moringa oleifera* plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthrax quinones in different solvent extracts as shown in Table 1.

Table 1: Phytochemicals screening of Moringa oleifera extract.

Sl	Test	Result
01	Alkaloides	+
02	Flavonoides	+
03	Saponin	+
04	Terpenoide	+
05	Tannin	+
06	Glycosides	+
07	Phytosterol	-
08	Proteins	+
09	Volatile oil	-
10	Reducing sugars	-

Where: + Present, - Absent

Antibacterial Activity

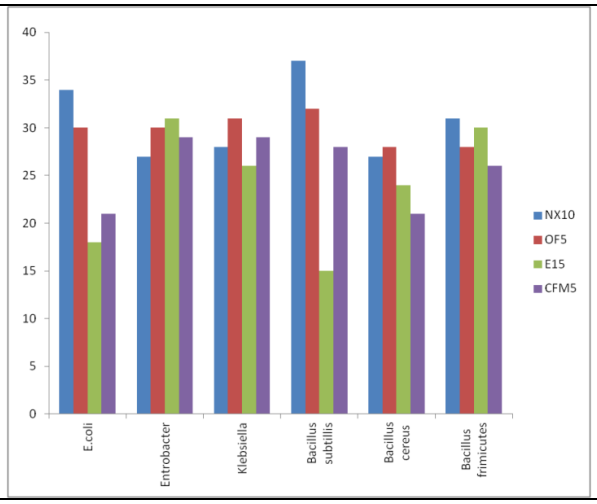
The results showed that the extracts of *Moringa oleifera* L. has a concentration dependent antibacterial activity with more sensitivity for gram positive bacteria than gram negative bacteria used in the extracts of *Moringa oleifera* showed considerable antibacterial activity at all the four concentrations 100, 75, 50, 25 mg/ml (Table 2). Table 3 shows the sensitivity of the tested bacteria to the standard antibiotics.

Table 2: The study of antibacterial activity of standard antibiotics using disk diffusion method.

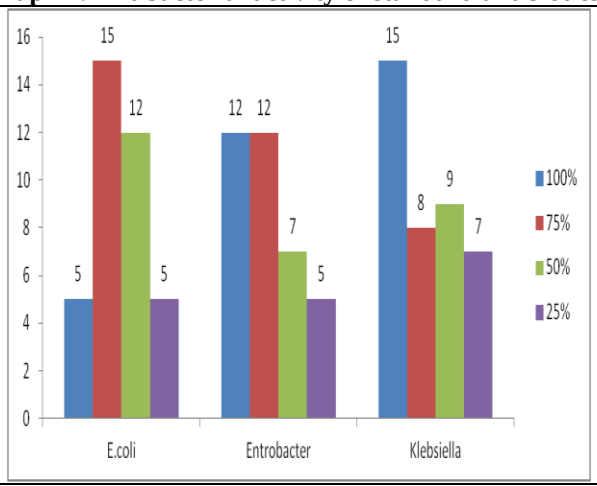
SI	Bacterial Strain	Bacterial Use	Zone of Inhibition(In mm)			
			NX10	OF5	E15	CFM5
1.	Gram Negative (-)	*E.coli*	34.00	30.00	18.00	21.00
		Entrobacter	27.00	30.00	31.00	29.00
		Klebsiella	28.00	31.00	26.00	29.00
2.	Gram Positive (+)	*Bacillus subtilis*	37.00	32.00	15.00	28.00
		Bacillus cereus	27.00	28.00	24.00	21.00
		Bacillus frimicutes	31.00	28.00	30.00	26.00

Table 3: The study of antibacterial activities of Moringa oleifera leaf extract using Disk Diffusion Method (Mean ± SE)

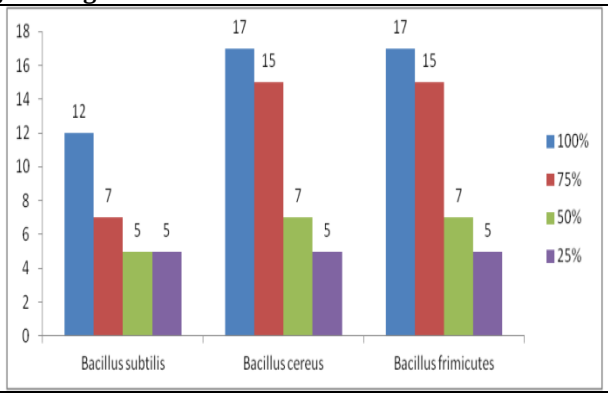
SI	Bacterial Strain	Bacterial Use	Zone of Inhibition(In mm)			
			100%	75%	50%	25%
1	Gram Negative (-)	*E. coli*	11.66±3.75	10.33±2.40	10.33±0.87	05.33±0.32
		Entrobacter	12.00±1.73	11.00±1.00	5.66±0.66	04.66±0.32
		Klebsiella	18.00±1.15	11.00±1.52	6.33±1.32	02.65±0.66
2	Gram Positive (+)	*Bacillus subtilis*	12.66±0.66	05.66±0.66	16.33±6.70	04.66±0.32
		Bacillus cereus	12.00±2.64	13.66±1.53	06.00±0.57	05.66±0.66
		Bacillus frimicutes	08.66±4.17	10.00±2.96	06.33±0.66	04.66±0.32



Graph 1: Antibacterial activity of standard antibiotics



Graph 2:- Antibacterial activity of *Moringa oliefera* gram negative bacteria



Graph 3:- Antibacterial activity of *Moringa oliefera*

gram positive bacteria

Fresh Leaf of *Moringa oleifera*

Dried Leaf of *Moringa oleifera*

Powder of *Moringa oleifera* ethanolic extract

4. DISCUSSION

Humans have long been using plants as dietary supplements as well as in the treatment of diseases. Currently, many plants are being annually investigated around the world in terms of therapeutic properties. A part of these researches focuses on determining the antimicrobial properties of medicinal plants. This has been confirmed due to the occurrence of problems such as microbial resistance and antibiotic complications. Low infectious doses of many food-borne pathogens require extensive research in the field of new pharmaceutical compounds with high bactericidal potential, which is very important to use oily compounds from plants to provide health and food safety in order to achieve this objective (Gill and Holley, 2006).

The present Study reveals that Moringa oleifera plant shows the presence of phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, proteins, saponins, tannins, terpenoids and anthrax quinones in different solvent extracts as shown in Table 1. Antibacterial activity of Moringa oleifera was seen against several bacteria namely *Escherichia Coli, Pseudomonas aeroginosa, Staphylococcus aureus, Proteus vulgaris, Streptococcus mutans, Bacillus subtilus and Staphylococcus epidermidis* (Napolean, *et. al.*, 2009). The ethanol leaf extract showed maximum activity against Streptococcus mutant and aqueous extract shows maximum activity against *Klebsiella* as shown in the Table 3. Alkaloids are naturally occurring chemical compounds containing basic nitrogen atoms.

They often have pharmacological effects and are used as medications and recreational drugs (Rhoades, *et. al*., 1979). Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes (Korkina, *et. al*., 1997). Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal sonedies and are under investigation for Antibacterial, Anti-neoplastic and other Pharmaceutical functions (Yamunadevi, *et. al*., 2011). Tannins have shown potential Antiviral, Antibacterial and Antiparasitic effects. Saponins cause hemolysis of red blood cells (Winter, *et. al*., 1993). The antibacterial activity was screened because of their great medicinal properties towards the pathogenic organisms. The medicinal plant *Moringa oleifera* showed good antibacterial activity against several organisms like *Staphylococcus aureus*, *Pseudomonas*, *Bacillus*, *Klebsiella*, and *E. coli* as supported by previous studies. *Moringa oleifera* extracts have rich amount of phytochemicals and antimicrobial properties; these can be of immense significance in therapeutic treatments. *Moringa oleifera* extract is found to have a great potential as antimicrobial compounds against numerous microorganisms. It can be reported *Moringa oleifera* could be a potential source of natural phytochemicals and antibacterial agents so further studies should be conducted to identify the active constituents responsible for this activity.

5. CONCLUSION

Moringa oleifera an important medicinal plant is one of the most widely cultivated species of the family Moringaceae. Pharmacologically reported that Different parts of it have been used for different human ailments, extracts showed varying degrees of antimicrobial and antifungal activity on the microorganism tested. Antibacterial activity of *Moringa oleifera* extracts on bacterial isolates showed that *Moringa oleifera* leaf ethanol (MLE) extract had the broadest spectrum of activity on the test bacteria. The result exhibited that MLE had antimicrobial activity against six bacterial isolates. *Moringa oleifera* is a good source of various phytochemicals like alkaloids, flavonoids, glycosides, saponins, and tannins. The antibacterial activity *Moringa oleifera* was clearly shown by the present study against various test organisms like *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus*, *Acetobacter*, *Citrobacter and Escherichia coli*. Moringa oleifera leaves to treat common medical conditions but a few use it for preventing and treating malnutrition. Presence of phytochemicals indicates possible preventive and curative properties of *M. oleifera* leaves.

The bactericidal effect of *M. oleifera* leaf extracts was determined against the isolated bacteria. It further discusses optimal conditions for the extraction of essential compounds responsible for the elimination of pathogenic bacteria. Further work is needed to carry out more pharmacological from the extracts in order to support antimicrobial activity of the M. oleifera. Our Study demonstrated that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms to carry out more pharmacological studies to support the use of *M. oleifera* as a medicinal plant.

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REFERENCES

1. Caceres A, Cabrera O, Morales O, *et al*. Pharmacological properties of Moringa Oleifera. 1: Preliminary screening for antimicrobial activity. J Ethnopharmacol, 1991; 33(3): 213–216.
2. Caceres A, Saravia A, Rizzo S, *et al*. Pharmacologic properties of Moringa Oleifera. 2: screening for antispasmodic, anti-inflammatory and diuretic activity. J Ethnopharmacol, 1992; 36(3): 233–237.
3. Eilert U, Wolters B, Nahrstedt A. The antibiotic principle of seeds of Moringa oleifera and Moringa stenopetala. Planta Med, 1981; 42(1): 55– 61.
4. Faizi S, Siddiqui BS, Saleem R, *et al*. Fully acetylated carbonate and hypotensive thiocarbamate glycosides from Moringa oleifera. Phytochemistry, 1995; 38(4): 957–963.
5. Faizi S, Siddiqui BS, Saleem R. Isolation and structure elucidation of new nitrile and mustard oil glycosides from Moringa oleifera and their effect on blood pressure. J Nat Prod, 1994; 57(9): 1256–1261.
6. Fakurazi S, Hairuszah I, Nanthini U. Moringa oleifera Lam prevents acetaminophen induced liver injury through restoration of glutathione level. Food Chem Toxicol, 2008; 46(8): 2611–2615.
7. Gill AO, Holley RA. Disruption of E. coli, Listeria monocytogenes and Lactobacillus sakei cellular membranes by plant oil aromatics. Int J Food Microbiol, 2006; 108: 1-9.
8. Guevara AP, Vargas C, Sakurai H, et al. An antitumor promoter from Moringa oleifera Lam. Mutat Res., 1991; 440(2): 181–188.
9. Iswar Chandra G, Shamim Q, Safwan AK, et al. Short Communication Evaluation of the Anthelmintic Activity of Moringa oleifera seeds. International Journal of Pharma Professional's Research, 2010; 1(1): 88–89.
10. Julia C. A study of nutritional and medicinal values of Moringa oleifera leaves from susaharan Africa: Ghana, Rwanda Senegal and Zambia, 2008; 1–113.
11. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol, 2003; 84(1): 105–108.

12. Korkina LG, Afanas'ev IB. Antioxidant and chelating properties of flavonoids. Adv Pharmacol. 1997; 38: 151–163.
13. Manguro LO, Lemmen P. Phenolics of *Moringa oleifera* leaves. Nat Prod Res., 2007; 21(1): 56–68.
14. Mehta K, Balaraman R, Amin AH, *et al.* Effect of fruits of *Moringa oleifera* on the lipid profile of normal and hypercholesterolemia rabbits. J Ethnopharmacol, 2003; 86(2–3): 191–195.
15. Mondal S, Chakraborty I, Pramanik M, et al. Structural studies of an immune enhancing polysaccharide isolated from mature pods (fruits) of Moringa oleifera (Sajina). Med Chem Research, 2004; 13: 390–400.
16. Murakami A, Kitazono Y, Jiwajinda S, et al. A thiocarbamate from the leaves of Moringa oleifera holds a strict structural requirement for inhibition of tumour promoter induced Epstein Barr virus activation. Planta Med, 1998; 64(4): 319–323.
17. Nadkarni KM. Indian Materia Medica. Bombay Popular Prakashan, 2009; 1: 811–816.
18. Napolean P, Anitha J, Emilin RR. Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam. Current Biotica, 2009; 3(1): 33–37.
19. Nitin GS, Bonde CG, Patil VV, et al. Analgesic activity of seeds of *Moringa oleifera* Lam. International Journal of Green Pharmacy, 2008; 108–110.
20. Pal SK, Mukherjee PK, Saha BP. Studies on the antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. Phytotherapy Research, 1995; 9(6): 463–465.
21. Palaniswamy U. Purslane-Drumsticks, Lokavani, 2004; 1: 23–25.
22. Pari L, Kumar NA. Hepato protective activity of *Moringa oleifera* on antitubercular drug-induced liver damage in rats. J Med Food, 2002; 5(3): 171–177.
23. Rhoades, David F. Evolution of Plant Chemical Defense against Herbivores. In: Rosenthal, editors. Herbivores: Their Interaction with Secondary Plant Metabolites. New York, USA: Academic Press, 1979; 41.
24. Roloff A, Weisgerber H, Lang U, et al. *Moringa oleifera* Lam. Weinheim, Germany: Enzyklopädie der Holzgewächse, 2009; 1–8.
25. Tahiliani P, Kar A. Role of *Moringa oleifera* leaf extract in the regulation of thyroid hormone status in adult male and female rats. Pharmacol Res, 2004; 41(3): 319–323.
26. Talhaliani P, Kar A. Review on *Moringa oleifera* Pharmacological Research, 2000; 41(3): 319–323.
27. Trapti R, Vijay B, Komal M, et al. Comparative Studies on Anthelmintic Activty of *Moringa oleifera* and *Vitex Negundo*. Asian J Research Chem, 2009; 2(2): 181–182.
28. Udupa SL, Udupa AL, Kulkarni DL. Studies on the anti-inflammatory and wound healing properties of *Moringa oleifera* and Aeglemarmelos. Fitoterapia, 1994; 65: 119–121.
29. Winter WP, Mason KT, Ford TD. Mechanism of saponin- induced red cell hemolysis: reexamination. Blood, 1993; 82(1): 461.
30. Yamunadevi M, Wesely EG, Johnson M. Phytochemical studies on the terpenoids of medicinally important plant Aervalanata L. using HPTLC. Asian Pacific Journal of Tropical Biomedicine, 2011; 1(2): S22–S225.