Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2014; 3(4): 202-208
Received: 29-09-2014
Accepted: 04-10-2014

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

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

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

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

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

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

Phytochemical analysis and antibacterial activity of *Maytenus emarginata* leaf and stem

Pooja Moteriya, Hemali Padalia, Tejas Rathod, Disha Menpara and Sumitra Chanda

Abstract
Traditional medicine is the main source of medical care for a great percentage of the population of the developing world. Medicinal plants have been a valuable source of natural active phytoconsituents that play an important role in treatment of many human diseases. In the present study, leaf and stem of *Maytenus emarginata* was evaluated for its antibacterial potential and polyphenol content. The extraction was done by individual cold percolation method using six different solvents viz., hexane, toluene, ethyl acetate, acetone, methanol and water. Phenolic and flavonoid content of different extracts was determined using Folin-ciocalteu assays and aluminium chloride colorimetric method respectively. Qualitative phytochemical analysis of the crude powder of *M. emarginata* leaf and stem was carried out for various phytoconstituents. The antimicrobial activity was evaluated by an agar well diffusion method against eight different pathogenic Gram negative and Gram positive bacteria. The extractive yield was maximum in aqueous and methanol extracts both in leaf and stem. Total phenol content was maximum in polar solvents methanol and acetone extracts both in leaf and stem. The solvent extracts of stem showed better antibacterial activity than a leaf. This may be because of the difference in the presence of phytoconsituents present in them.

Keywords: *Maytenus emarginata*, Antibacterial activity, Total phenol content, leaf, stem, phytochemical analysis.

1. Introduction
Herbal medicine, a component of ethno-medicine, is as old as the history of man and spans all cultures. Medicinal plants have been useful in the development of new drugs and continue to play an invaluable role in the drug discovery process [12]. Herbal medicines have already formed the basis of therapeutic use in developing countries, but recently have also seen an increase in the use of herbal medications in the developed world as well. This is mainly because these herbs/plants are relatively cheap, easily available and their uses are dependent on ancestral experience.

India is a varietal emporium of medicinal plants and is one of the richest countries in the world as regards to genetic resources of medicinal plants. All known types of agro climatic, ecological and edaphic conditions are met within India. The biogeographic position of India is unique which makes India rich in all the three levels of biodiversity [24]. Some studies focusing on the investigation of traditional Indian medicinal plants have resulted in the identification of new sources of therapeutic agents [3]. Medicinal and aromatic plants form a numerically large group of economically important plants which provide basic raw materials for medicines, perfumes, flavors and cosmetics. These plants and their products not only serve as valuable source of income for small holders and entrepreneurs but also help the country to earn valuable foreign exchange by way of export.

Plants are recognized for their ability to produce a wealth of secondary metabolites and mankind has used many species for centuries to treat a variety of diseases [12]. The curative properties of herbs lie in secondary metabolites with *in situ* functions including growth regulation, inter and intra-specific interactions and defense against predators and infections. Many of these natural products have been shown to present interesting biological and pharmacological activities and are used as chemotherapeutic agents or serve as the starting point in the development of modern medicines [45, 46].

Herbs are safe, less toxic, economical and a reliable key natural resource of drugs all over the world.

Medicinal and healing properties of herbs are closely related to their chemical components which are classified into some major groups like alkaloids, essential oils, steroids, saponins, tannins etc. and all of them show various pharmacological activities [1, 26]. They are also reported to show very good antibacterial activities [14, 20]. Phytochemicals such as flavonoids, tannins, steroid and alkaloids have anti-inflammatory effects [4].

Phenolic compounds are secondary metabolites which are synthesized in plants. They possess beneficial biological properties such as antioxidant, anti-apoptotic activities, anti-aging, anticarcinogen, anti-inflammation, cardiovascular protection, improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation activity. Most of these biological action have been attributed to their intrinsic reducing capabilities [15]. Moreover, antibacterial activity of polyphenols against Gram positive and Gram negative bacteria have been reported in various plant species [29, 37, 40]. Polyphenol also serve in plant defense mechanisms to counteract reactive oxygen species in order to survive, prevent molecular damage and disrupt by microorganism, insect and herbivores [44].

Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern [48]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [5]. Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind. In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of disease, but are also faced with adulteration and side effects. Therefore, there is a need to search new safe infection fighting strategies to control microbial infection.

Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. There is a continuous and an urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases [36]. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity. Thus, plants can be investigated for their antimicrobial efficacy and polyphenolic content.

Maytenus emarginata (Willd) D. Hou. belonging to the family Celastraceae, is an evergreen tree that generally grows as small trees, bushes or lianas and has resinous stems and leaves. They tolerate various types of stresses of the desert, locally known as vickado, "Kankero" in Hindi, "Thorny staff tree" in English. Synonyms of this plant are *Celastrus emarginatus* Willd. *Gymnosporia emarginata* (Willd) Thw. *Gymnosporia montana* (Roth) Benth. Traditionally, species of *Maytenus* have been used for fever, asthma, rheumatism and gastrointestinal disorders worldwide. Some biomolecules from *Maytenus* species has been reported to be active against HIV-Protease [17] Carcinoma and leukemia [41] and MDR (Multi Drug Resistance) [43]. Various parts of this plant contain immense medicinal properties such as shoots of the plant help for mouth ulcer [38]. The bark is ground to a paste and applied with mustard oil to kill lice in the hair. Pulverized leaves are given in milk to children as a vermifuge [23]. A decoction of the leaf twigs is used as a mouthwash to relieve toothache. Ash of leaves is used to heal up sores and wound gives a cooling effect. The leaves are burnt and mixed with ghee to form an ointment used to heal sores [33]. The tender leaves are chewed raw in the treatment of jaundice. The fruit is used in medicines to purify blood [2].

In the present work, various solvent extracts of leaf and stem of *Maytenus emarginata* were evaluated for its phenol and flavonoid content and antibacterial potential.

2. Material and methods

2.1 Collection of plant materials

The leaf and stem of *Maytenus emarginata* (Willd) D. Hou. was collected in the month of August 2013 from Saurashtra University Campus, Rajkot, Gujarat, India. The plant was compared with voucher specimen (voucher specimen PSN 118) deposited at Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. They were thoroughly washed, separated and dried under shade. The dried plant parts (leaf and stem) were crushed to fine powder and stored in air tight bottles which were later used for further studies.

2.2 Extraction

The dried powder of the leaf and stem of *M. emarginata* was extracted individually by the cold percolation method [30] using different organic solvents like petroleum ether (PE), toluene (TO), ethyl acetate (EA), acetone (AC), methanol (ME) and water (AQ). Ten grams of dried powder were added to 100 ml of hexane in a conical flask, which was plugged with cotton wool and kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 min. Supernatant was collected and the solvent was evaporated. The residue was then added to 100 ml of solvents (toluene, ethyl acetate, acetone, methanol and water) in different conical flasks, which were plugged with cotton wool and kept on a rotary shaker at 120 rpm for 24 h. After 24 h, the extract was filtered with 8 layers of muslin cloth and centrifuged at 5000 rpm for 10 min. The supernatant was collected and the solvents were evaporated; the dry extract was stored at 4 °C in airtight bottles. The extracts were weighed to obtain the extraction yield.

2.3 Qualitative phytochemical analysis

The crude powder of leaf and stem of *M. emarginata* was subjected to qualitative phytochemical analysis [16, 25] to identify the presence or absence of different phytoconstituents.

2.4 Quantitative phytochemical analysis

Total phenol and total flavonoid content were estimated in all the solvent extracts of *M. emarginata* leaf and stem.

2.5 Determination of total phenol content

The amount of total phenol content of different solvent extracts of *M. emarginata* leaf and stem was determined by Folin- ciocalteu's reagent method [27]. The extract (0.5) ml and 0.1 ml of Folin-ciocalteu's reagent (0.5N) were mixed and the mixture was incubated at room temperature for 15 min. Then, 2.5 ml of sodium carbonate (2M) solution was added and further incubated for 30 min at room temperature and the absorbance was measured at 760 nm (Systronic, India), against a blank sample. The calibration curve was made by preparing

Gallic acid (10 to 100 µg ml^{-1}) solution in distilled water. Total phenol content is expressed in terms of Gallic acid equivalent (mg g^{-1} of extracted compounds).

2.6 Determination of total flavonoid content

The amount of flavonoid content of different solvent extracts of *M. emarginata* leaf and stem was determined by aluminium chloride colorimetric method [10]. The reaction mixture (0.3 ml) consisted of 1.0 ml of sample (1 mg ml^{-1}), 1.0 ml methanol, 0.5 ml of aluminium chloride (1.2%) and 0.5 ml potassium acetate (120 mM) and was incubated at room temperature for 30 min. The absorbance of all samples was measured at 415 nm using a digital spectrophotometer (Systronic, India), against a blank sample. The calibration curve was made by preparing quercetine (5 to 60 µg ml^{-1}) solution in methanol. The flavonoid content is expressed in terms of standard equivalent (mg g^{-1}) of extracted compound.

2.7 Antimicrobial susceptibility test

Test microorganisms

The microorganisms used were obtained from the National Chemical Laboratory, Pune, India. The microorganisms were maintained at 4 °C. The Gram-positive bacteria studied were *Bacillus cereus* (BC) ATCC11778, *Staphylococcus aureus 2* (SA2) ATCC29737, *Listeria monocytogenes* (LM) ATCC19112 and *Corynebacterium rubrum* (CR) ATCC14898. The Gram-negative bacteria were *Escherichia coli* (EC) NCIM2931, *Pseudomonas aeruginosa (PA)* ATCC27853, *Klebsiella pneumoniae* (KP) NCIM2719 and *Salmonella typhimurium* (ST) ATCC23564.

2.8 Antimicrobial activity (Agar well diffusion assay)

In vitro antimicrobial activity of different solvent extracts of leaf and stem of *M. emarginata* was determined by standard agar well diffusion assay [32]. Mueller Hinton agar and Sabouraud dextrose agar media were used for antibacterial and antifungal activity respectively. Molten Mueller Hinton agar/Sabouraud dextrose agar (40-42 °C) were seeded with 200 µl of inoculums (1 × 10^8 cfu/ml) and poured into Petri dishes. The media were allowed to solidify and wells were prepared in the seeded agar plates with the help of a cup borer (8.5 mm). Different extracts were dissolved in 100% DMSO at a concentration of 20 mg/ml, from this 100 µl of different extracts were added into the sterile 8.5 mm diameter well. The plates were incubated at 37 °C and 28 °C for 24 and 48 h for bacteria and fungi, respectively. DMSO was used as a negative control. Antibacterial activity was assayed by measuring the diameter of the zone of inhibition formed around the well in millimeters. The experiment was done in triplicate and the average values were calculated for antibacterial activity.

3. Results and discussion

3.1 Extractive yield

Efficiency of extraction is an important step involved in the discovery of bioactive components from plant material. In the present work, dry powder of leaf and stem of *M. emarginata* was extracted with solvents of different polarity like petroleum ether, toluene, ethyl acetate, acetone, methanol and water by individual cold percolation method. The extractive yield varied amongst the different parts and also amongst different solvents used. In leaf, maximum extractive yield was in aqueous extract. Amongst all organic solvents, highest extractive yield was in polar solvent ME; but less in another polar solvent AC extract. The extractive yield of nonpolar solvent PE extract was less but more than semi polar solvent extracts EA and TO (Fig. 1A).

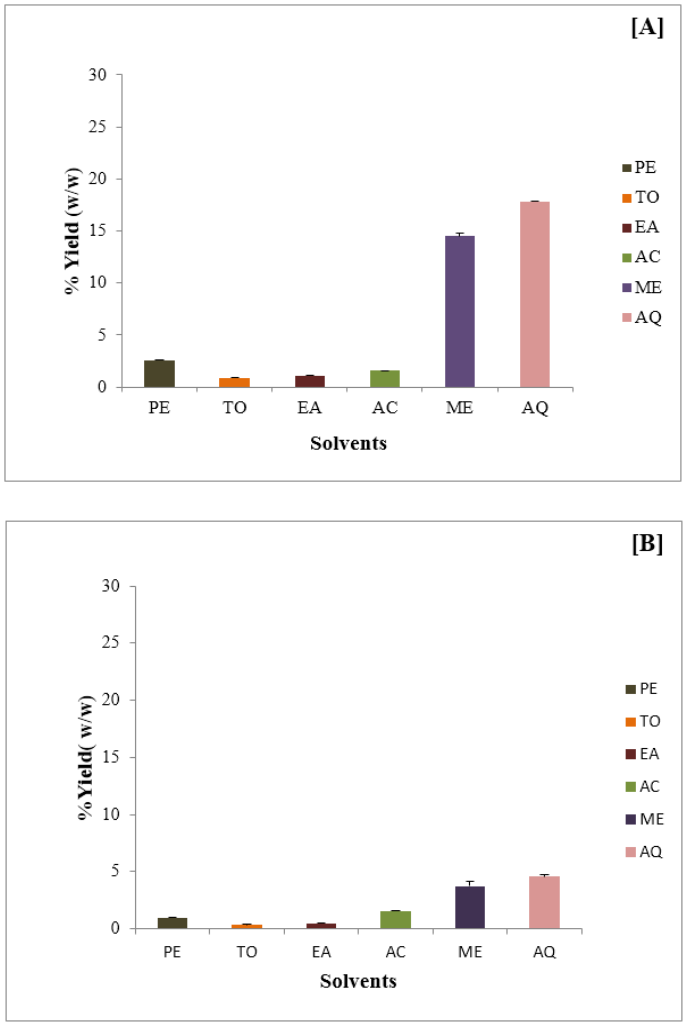


Fig 1: Extractive yield of different solvents extracts of *M. emarginata* in leaf [A] and stem [B]

The extractive yield of *M. emarginata* stem in all the solvents was distinctly less than that of leaf (Fig. 1B). Amongst all the six solvents used, maximum extractive yield was in ME extract and aqueous extract. The yield in another four solvents was quite less. A distinct effect of solvent was envisaged. In *M. emarginata*, leaf and stem, maximum yield was in polar solvent methanol; the yield in acetone extract was less though it is a polar solvent. Very less yield was obtained in nonpolar and semi polar solvents. Different solvents give different yield depending upon the phytoconstituents present in them and their solubility in a particular solvent. Hence, choice of a solvent for extraction is important as also reported by Park and Chanda S *et al.*, Kaneria M *et al.*, Kaneria M *et al.*, Park EJ *et al.*, [8, 21, 22, 31].

3.2 Qualitative phytochemical analysis

The results of qualitative phytochemical analysis of the crude powder of *M. emarginata* leaf and stem are shown in Table 1. The leaf had maximum amount of flavonoids content followed by tannins; steroids, triterpens and cardiac glycosides were present in trace amount while saponins and phlobatanins were absent. The stem had maximum amount of tannins, while flavonoid, triterpens and alkaloids were present in minimal

amount; phlobatanins, steroids, saponins and cardiac glycosides were absent. Plants rich in flavonoids or tannins are reported to show good antibacterial activity [43].

Table 1: Qualitative phytochemical analysis of *M. emarginata* leaf and stem

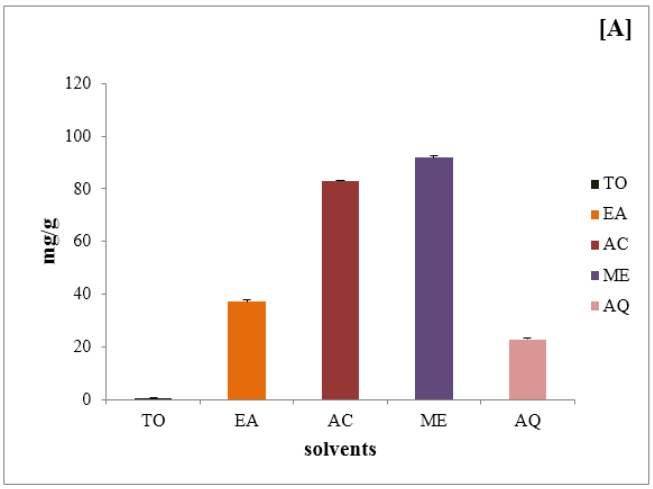
NO.	Test	leaf	stem
1	Flavonoids	+++	+
2	Tannins	++	+++
3	Phlobatannins	-	-
4	Saponins	-	-
5	Steroid	++	-
6	Cardiac glycosides	+	-
7	Triterpenes	+	-
8.	Alkaloids		
	(1)Mayer's	+	+
	(2) Dragondroff's	+	+
	(3)Wagner's	-	+

Phytochemicals present in less (+), moderate (++) and high (+++) amount; absent (-)

3.3 Quantitative phytochemical analysis

Total phenol content

Total phenol content (TPC) of different solvent extracts of *M. emarginata* is shown in Fig. 2. In *M. emarginata* leaf, TPC was highest in polar solvent ME extract (Fig. 2A). But there was not much difference between TPC of ME extract and AC extract. TPC was very much less in semi polar solvent extracts TO and EA as compared to polar solvent extracts (AC and ME). TPC of TO extract was negligible. The TPC of AQ extract was also less. In *M. emarginata* stem, both polar solvents AC and ME extracts had maximum TPC (Fig. 2B). In both semi polar solvent extracts, TPC was less than polar solvent extracts; TO extract had considerably less TPC than EA extract. The TPC of AQ extract was almost equal to that of EA extract.



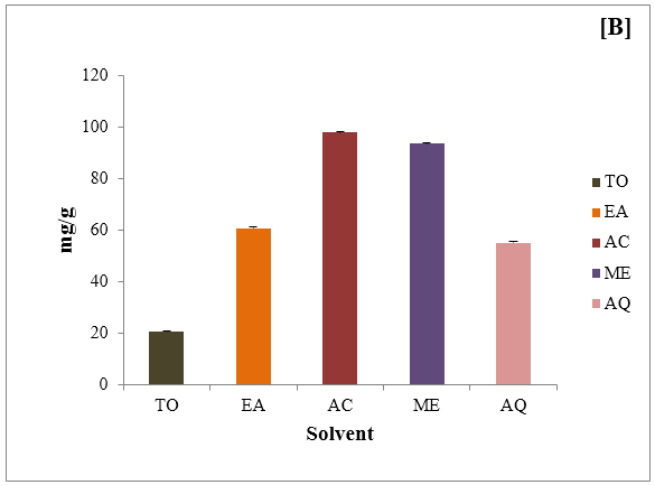


Fig 2: Total phenol content of different solvents extract of *M. emarginata* leaf [A] and stem [B]

In *M. emarginata*, AC and ME extracts of both leaf and stem had maximum TPC, with very slight difference in each part. On the other hand, both TO and EA extracts of stem had more TPC than leaf. When AQ extracts of both leaf and stem are compared, AQ extract of leaf had lower TPC. Overall, it can be concluded that maximum TPC was in AC extract of stem. It is established fact that the extraction yield of phenols is greatly influenced by the polarity of the solvent [18, 34, 42].

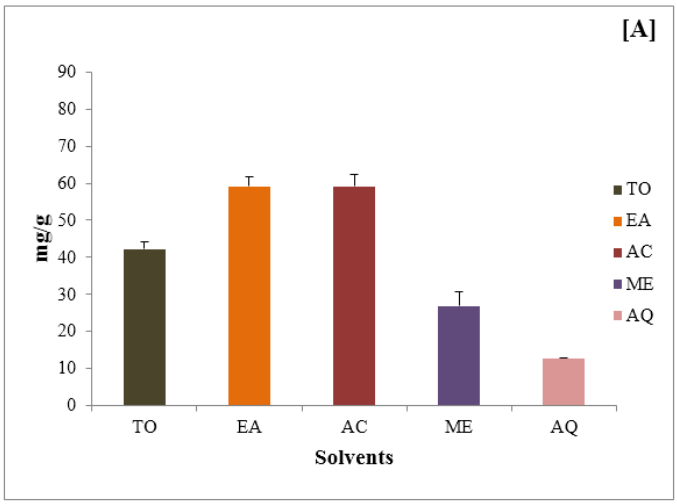
Total flavonoid content

Total flavonoid content (TFC) of different solvent extracts of *M. emarginata* is shown in Fig. 3. In leaf, EA and AC extract had almost some TFC followed by TO and ME extract respectively. AQ extract had lowest TFC (Fig. 3A). In stem, maximum TFC was in the TO extract and minimum in ME extract. AQ extract had a very less amount of flavonoid content; polar solvent extracts ME and AC also had a very low amount of flavonoid content (Fig. 3B). When TFC of both leaf and stem are compared, maximum TFC was in EA and AC extracts of leaf (Fig. 3A).

3.4 Antibacterial activity

The antibacterial activity of different solvent extracts of *M. emarginata* leaf and stem against Gram positive bacteria is shown in Fig. 4. Different solvent extracts of stem showed more activity than leaf. AQ extract of leaf and stem did not show any antibacterial activity. In leaf, *B. cereus* was inhibited by PE extract alone and *S. aureus* 2 was inhibited by PE and AC extracts. None of the other solvent extracts showed activity against these two Gram positive bacteria. *L. monocytogenes* and *C. rubrum* were most resistant bacteria because they were not inhibited by any of the solvent extracts (Fig. 4A). In stem, all the five solvent extracts inhibited *B. cereus* and *S. aureus2*; maximum antibacterial activity was shown by AC extract. Here also, *L. monocytogenes* and *C. rubrum* were the most resistant bacterial strains (Fig. 4B).

The antibacterial activity of different solvent extracts of *M. emarginata* leaf against Gram negative bacteria is shown in Fig. 5. S. *typhimurium* and *P. aeruginosa* were most resistant bacteria in leaf (Fig. 5 A) and all the four tested Gram negative bacteria were resistant in stem. AQ extract inhibited *E. coli* and *K. pneumoniae*. *E. coli* was also inhibited by PE and TO extract.



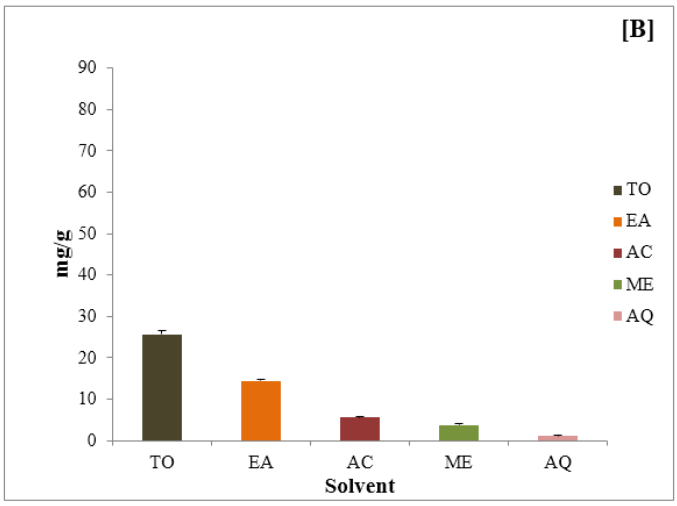
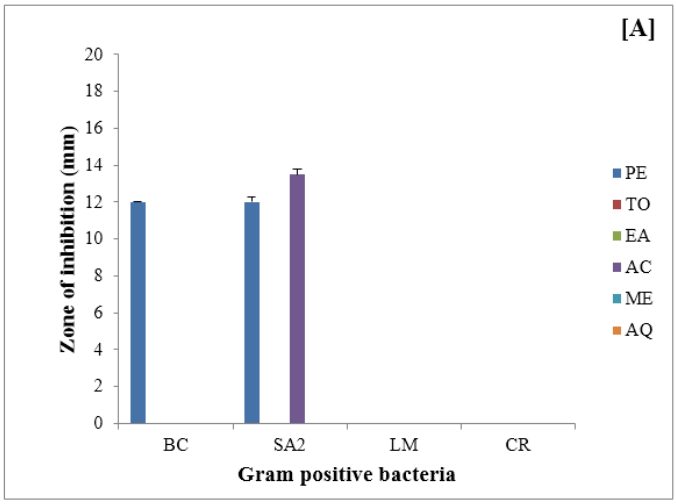


Fig 3: Total flavonoid content of different solvents extract of *M. emarginata* leaf [A] and stem [B]



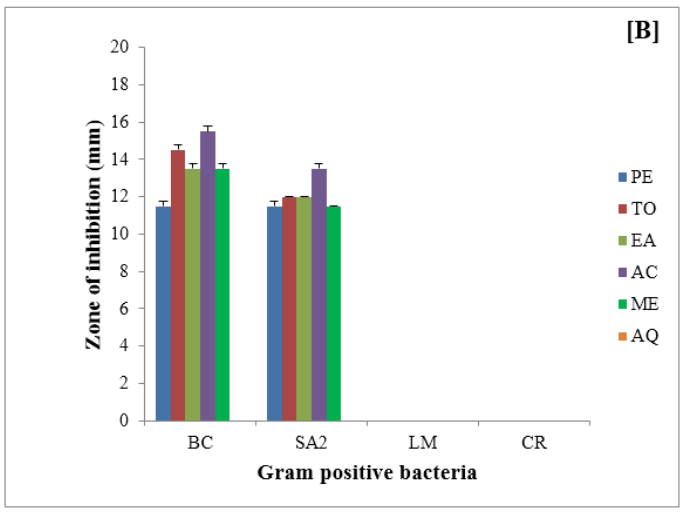


Fig 4: Antibacterial activity of different solvent extracts of *M. emarginata* leaf (A) and stem (B) against Gram positive bacteria

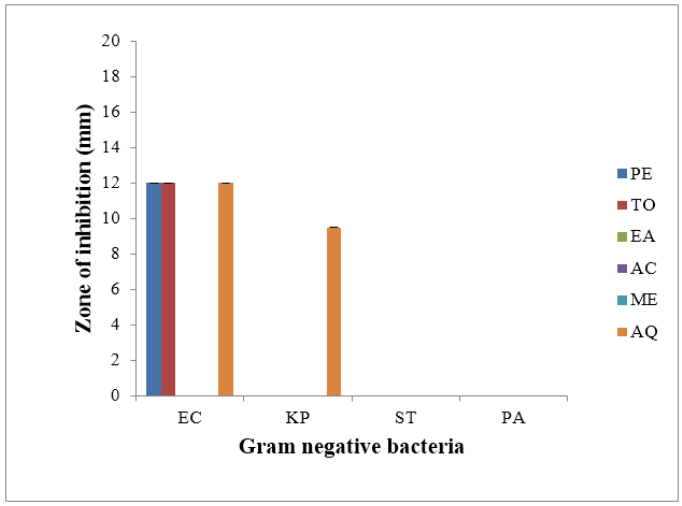


Fig 5: Antibacterial activity of different solvent extracts of *M. emarginata* leaf against Gram negative bacteria.

From the above results, it can be concluded that in *M. emarginata*, amongst the two parts, leaf and stem, the solvent extracts of stem showed best antibacterial activity against Gram positive bacteria and amongst different solvent extracts, acetone extract showed maximum inhibitory activity. They did not show any activity against Gram negative bacteria. The solvent extracts of leaf showed very poor activity towards Gram positive and Gram negative bacteria. In *M. emarginata*, there was a direct correlation between TPC and antibacterial activity. The plant part stem had maximum TPC in AC extract, which also showed maximum antibacterial activity.

The results once again prove that crude plant extracts show different levels of antibacterial activity. It depends on the part of the plant, the solvent used and the bacterial strains under investigation. The difference in the activity of plant parts is because they contain different phytoconstituents in different concentrations. The difference in susceptibility of Gram positive and Gram negative bacteria towards plant extracts is because of the difference in their cell wall structure. The antibacterial activity shown by different solvent extracts may be because of the various phytoconsituents present in them.

They may act alone or in combination to inhibit bacterial

growth. The plant extracts were rich in flavonoids and tannins all of which are known for their antibacterial effects [6, 13, 19]. Finally, it can be stated that natural plant extracts can emerge as new source of antimicrobial agents against multi drug resistant microorganisms. However, more studies are needed to be done before using them as antimicrobics. Fractionation of potent extracts, followed by appropriate structure elucidation using various spectral analysis should be done which may lead to isolation and identification of novel lead compounds.

4. Acknowledgments
The authors thank Prof. S. P. Singh, Head, Department of Biosciences (UGC-CAS), Saurashtra University, Rajkot, Gujarat, India for providing excellent research facilities. One of the author (TR) thank UGC, New Delhi for providing meritorious Junior Research Fellowship.

5. References

1. Aiyegoro AO, Okoh AI. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. BMC Complementary and Alternate Medicine 2010; 10:21.
2. Agrawal M, Nag TN. Seasonal variations in flavonoid content in *Maytenus emarginata* (Willd.) Ding Hou. Journal of Indian Botanical Society 2009; 88(3, 4):177-180.
3. Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Journal of Ethnopharmacology 2001; 74:113-123.
4. Akindde AJ, Adeymi OO. Anti-inflammatory of the aqueous leaf extract *Byrsocarpus coccineus*. Fitoterapia 2007; 78:25-28.
5. Bandow JE, Brotz HL, Labischinski H, Heicker M. Proteomic approach to understanding antibiotics action. Antimicrobial Agents and Chemotherapy 2003; 47:945-955.
6. Bhat RS, Sooad AD. Phytochemical constituents and antibacterial activity of some green leafy vegetables. Asian Pacific Journal of Tropical Biomedicine 2014; 4(3):189-193.
7. Chanda S, Dudhatra S, Kaneria M. Antioxidative and antibacterial effects of seeds and fruit rind of nutraceutical plants belonging to Fabaceae. Food and Function 2010; 1:308-315.
8. Chanda S, Kaneria M. Optimization of conditions for the extraction of antioxidants from leaves of *Syzygium cumini* L. using different solvents. Food Analytical Methods 2012; 5:332-338.
9. Chanda S, Amrutiya N, Rakholiya K, Evaluation of antioxidant properties of some Indian vegetable and fruit peels by decoction extraction method. American Journal of Food Technology 2013: 8(3):173-182.
10. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in Propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 2002; 10:178-182.
11. Chanudom L, Phuangthip B, Khwanchuea Tangpong J. Antioxidant and antimicrobial activities of aqueous and ethanol crude extracts of 13 Thai traditional plants. International Journal of Current Microbiology Applied Science 2014; 3(1):549-558.
12. Cragg GM, Boyd MR, Khanna R, Kneller R, Mays TD, Mazan KD *et al*. International collaboration in drug discovery and development: the NCI experience. Pure and Appllied Chemistry 1999; 71(9):1619- 1633.
13. Cushnie TPT, Lamb AJ. Antimicrobial activity of flavonoids Review. International Journal of Antimicrobial Agents 2005; 26:343–356.
14. Doughari J, Manzara S. *In vitro* antibacterial activity of crude leaf extracts of *Mangifera indica* Linn. African Journal of Microbiology Research 2008; 2:67–72.
15. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. International Journal of Molecular Bioscience 2007; 8(9):950-998.
16. Harborne JB. Phytochemical Methods 2nd Ed. London: Chapman & Hall 1973.
17. Hussein G, Yashiva H, Nakamur NH. Inhibitory effects of Sudanese plant extract on HIV-1 replication and HIV-1 protease. Phytotherapy Research 1991; 13:31-36.
18. Jakopic J, Robert V, Stampar F. Extraction of phenolic compounds from green walnut fruits in different solvents. Acta Agriculturae Slovenica 2009; 93(1):11-15
19. Johanna WI. Spicing up a vegetarian diet: chemopreventive effects of phytochemicals. American Journal of Clinical Nutrition 2003; 78:579-583.
20. Jaberian H, Piri K, Nazari J. Phytochemical composition and *in vitro* antimicrobial and antioxidant activities of some medicinal plants. Food Chemistry 2013; 136:237–244.
21. Kaneria M, Chanda S. Evaluation of antioxidant and antimicrobial properties of *Manilkara zapota* L. (chiku) leaves by sequential Soxhlet extraction method. Asian Pacific Journal of Tropical Medicine 2012; S1526-S1533.
22. Kaneria M, Chanda S. The effect of sequential fractionation technique on the various efficacies of pomegranate (*Punica granatum* L.). Food Analytical Methods 2012; 6:164-175.
23. Kothari MJ, Lonhe AN. Ethanobotany in human health care of Chikhaldara, Amravati state, India. Ethanobotany and medicinal plants of Indian subcontinent. Scientific Publishers (India) Jodhapur 2000, 273-281.
24. Krishnaraju AV, Rao TNV, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Biological screening of medicinal plants collected from Eastern ghats of India using *Artemia salina* (brine shrimp test). International Journal of Applied Science and Engineering 2006; 4(2):115-125.
25. Menpara D, Chanda S, Phytochemical and phamacognostic evalution of leaves of *Pongamia pinnata* L. (Fabaceae). Phcognocy Communication 2014 (In Press).
26. Mbaebie BO, Edeoga HO, Afolayan AJ. Phytochemical analysis and antioxidants activities of aqueous stem bark extract of *Schotia latifolia* Jacq. Asian Pacific Journal of Tropical Biomedicine 2012, 118-124.
27. McDonald S, Prenzler PD, Autolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chemistry 2001; 73:73-84.
28. Miliauskas G, Venskutonis PR, Van BTA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chemistry 2004; 85:231-237.
29. Padam BS, Tin HS, Chye FY, Abdullah MI. Antibacterial and antioxidative activities of the various solvent extracts of Banana (*Musa paradisiaca* cv.

Mysore). Inflorescences Journal of Biological Sciences 2012; 12:62-73.
30. Parekh J, Chanda S. *In vitro* antibacterial activity of the crude methanol extract of *Woodfordia fruticosa* kurz. Flower (Lythraceae). Brazilian Journal of Microbiology 2007; 38:204-2007.
31. Park EJ, Jhon DY. The antioxidant, angiotensis converting enzyme inhibition activity, and phenolic compounds of bamboo shoot extracts. LWT-Food Science and Technology 2010; 43:655-659.
32. Perez C, Paul M, Bazerque P. An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicine Experimentalis 1990; 15:113–115.
33. Pullaiah T. Encyclopedia of World Medicinal Plants, Sal. Paratyphi. Regency Publication, Edn 1, New Delhi, 2006, 1316-1317.
34. Rakholiya KD, Kaneria MJ, Chanda SV. Mango pulp: A potential source of natural antioxidant and antimicrobial agent. In: Medicinal Plants: Phytochemistry, Pharmacology and Therapeutics – Vol. 3. Ed. Gupta VK, Daya Publishing House, New Delhi 2014, 253-284.
35. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. Journal of Ethnopharmacology 2005; 100:80-84
36. Rojas R, Bustamante B, Bauer J, Fernandez I, Alban JL. Antimicrobial activity of selected Peruvian medicinal plants. Journal of Ethnopharmacology 2003; 88:199-204.
37. Scalbert A, Johnson IT, Saltmarsh M. Polyphenols: antioxidants and beyond. American Journal of Clinical Nutrition 2005; 81:215S-217S.
38. Spivey AC, Weston M, Woodhead S. Celastraceae sesquiterpenoids: biological activity and synthesis. Chemical Society Review 2002; 31:43-59.
39. Suleiman MM, Mcgaw LJ, Naidoo V, Ellof JN. Detection of antimicrobial compounds by bioautography different of extract of leaves of selected South African tree species. The African Journal of Traditional, Complementary and Alternative medicines 2010; 7(1):64-78.
40. Taguri T, Tanaka T, Kouno I. Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. Biological and Pharmaceutical Bulletin 2006; 29: 2226-2235.
41. Tin-wa MNR, Farnswarth HHS, Fong RN, Blomster J, Tojanek DI, Abraham GJ *et al*. Ethanolic extract of *M. senegalensis* demonstrated cytotoxic effects against carcinoma in cell cultures and Leukemia in mice. Journal of Natural Products 1971; 34:79-87.
42. Turkmen N, Sari F, Velioglu YS. Effect of extraction solvents on concentration and antioxidant activity of black and black mate polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chemistry 2006; 99:838-841.
43. Tereschuka ML, Rieraa MVQ, Castrob GR, Abdala LR, Antimicrobial activity of flavonoids from leaves of *Tagetes minuta.* Journal of Ethnopharmacology 1999; 56(3):227-23.
44. Vaya J, Belinky PA, Aviram M. Antioxidant constituents from licorice roots: Isolation, structure elucidation and antioxidative capacity toward LDL oxidation. Free Radical Biology and Medicine 1997; 23(2):302–313.
45. Verpoorte R. Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. Drug Discovery Today 1998; 3:232-238.
46. Verpoorte R. Pharmacognosy in the new millennium: lead finding and biotechnology. Journal of Pharmacy and Pharmacology 2000; 52:253-262.
47. Vilegas W, Sanommiya M, Rastrelli L, Pizza C. Isolation and structure elucidation of two new flavonoids glycosides from the infusion of *Maytenus aquifolium* leaves. Evaluation of the antiulcer activity of the infusion. Journal of Agricultural and Food Chemistry 1999; 47(2):403-406.
48. Westh H, Zinn CS, Rosdahl VT. An international multicenter study of antimicrobial consumption and resistance in *Staphylococcus aureus* isolates from 15 hospitals in 14 countries. Microbial Drug Resistance 2004; 10:169-176.
49. Zulkefli HN, Mohamad J, Abidin NZ, Antioxidant activity of *Tinospora crispa* and *Tabernaemontana corymbosa.* Sains Malaysiana 2013; 42(6):697-706.