Science Arena Publications
Specialty Journal of Biological Sciences
ISSN: 2412-7396
Available online at www.sciarena.com
2018, Vol, 4 (2): 1-6

Antibiotic Potency of Myrtus Communis Leaves, Stems and Flowers' Extracts against Bacterial Pathogens Responsible for Diarrhoea

Javid Ali[1], Muhammad Siddique Afridi[2*], Shamsur Rehman[1], Arshad Hussain[1], Ziaurehman[1]

[1]Food Technology Department, Pakistan Council of Scientific and Industrial Research Laboratories Complex Peshawar, Khyber Pakhtunkhwa, Pakistan
[2] Medicinal Botanical Department, Pakistan Council of Scientific and Industrial Research Laboratories Complex Peshawar, Khyber Pakhtunkhwa, Pakistan

***Corresponding Author:**
Email: afridi_mdk @ hotmail.com

Abstract : *The main purpose of this study was to find out the antibiotic potency of extracts of Myrtus communis' leaves, stems and flowers prepared in different solvents (water, ethanol, methanol and n-hexane). The qualitative phytochemical analyses were carried out by standard methods. Agar disc diffusion method was employed to investigate the antibacterial activities of the diarrhoea disease pathogens. The results showed that the methanol extracts of Myrtus communis leaves have more phytochemicals, and showed the highest antibacterial activities against the tested bacteria as compared to the stem and flower extracts. The study concluded that leaves, stems and flowers of this plant have potent phytochemical (carbohydrates, tannins, phenols, saponins, alkaloids, glycosides, flavonoids and terpenoids) and antibacterial activities against Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella Typhi, Shigella sonnei, Vibrio cholerae, Shigella dysenteriae and Shigella flexneri.*

Keywords: *Myrtus Communis, Solvent Extracts, Antibacterial Activity, Diarrhea, Herbal Medicine*

INTRODUCTION

Plants produce numerous bioactive compounds, they are a rich source of medicine (Farhana et al., 2009). Traditional folk medicine is one of the primary health care strategies in numerous budding countries (Fransworth, 1993; Houghton, 1995). Medicinal plants are extensively utilized in the conventional health system, and their positive results are corrigible as well as documented (Dubey, et al., 2004).

One of the major community health issues in the budding countries is diarrhea, and it is estimated that 3.3 – 6.0 million kids die annually because of this disease. The major epidemic and sporadic diarrhea both in kids and adults includes Escherichia coli, Salmonella sp., Pseudomonas sp., Staphylococcus aureus, Klebsiella sp., Shigella sp., Proteus sp., and Vibrio cholerae (WHO, 1985). Recently, it has been reported that many human pathogenic bacteria have developed resistance aligned with allopathic medicine. So, alternative medicines are

needed to be searched due to the side effects and inefficacy of allopathic drugs (Vickers and Zollman, 1999; De Smet, 2002; Dawson, 2005).

Myrtus communis (M. communis) is distributed in Asia, Africa, America and Europe (Haydar et al., 2012). In traditional medicine, this plant is used in lung and digestive disorders, as well as antiseptic, anti-inflammatory and mouthwash (Elif et al., 2014). Pilot scale assessment of the indigenous plants practicing in ethno- medicine for numerous biological and pharmacological activities is the first primary tests in the separation, purification and characterization of bioactive compounds for medicinal product development.

This is the need of the day to isolate the bioactive compounds from herbs and utilize them for health problems. The present research project was initiated to analyze the phytochemical and antibiotic potential of M. communis stem, flower and leaves extracts.

Material and Methods

Plant materials

The leaves, flowers and stems of M. communis were collected from Medicinal Botanical Garden of Pakistan Council of Scientific and Industrial Research [PCSIR] Laboratories Complex Peshawar-Pakistan during the vegetation period. The collected plant parts were shade dried on filter paper sheets for 10 days. These were grinded in a laboratory mill to the powder form.

Extracts preparation

Twenty grams of leaves, flower and stem powder were soaked at room temperature for 10 days in 1L of conical flask in 500 ml of methanol, n-hexane and ethanol solvent (each). While for the aqueous extract, twenty gram leaves, stem and flower powder were soaked for 15 min in hot water (500 ml) on a hot plate. The whole extraction process was repeated three times. At the end, the filtration was carried out through Watman No. 1 filter paper. The solvent was evaporated by Vacuum Rotary Evaporator (Buchi Rotavapor R-200, Buchi Heating Bath B-490, Switzerland) to get a dehydrated extract. The extracted materials were kept at 4 °C in a cooled incubator (Gallenkamp, England) for further analysis.

Qualitative Phytochemical Analysis

The preliminary qualitative phytochemical tests were investigated to detect the main bioactive compounds in the leaves, stem and flower extracts of M. communis as described in the literature (Trease and Evans 2002; Harborne 1984).

Collection and Preparation of Bacterial Culture

The bacteria were collected from Microbiology Section of PCSIR Laboratories Complex Peshawar Pakistan (Table 2). Trypticase Soy Agar (TSA) slants were prepared for each microorganism and stored at 4°C. Working cultures of each microbe were individually and aseptically inoculated in Trypticase Soy Broth (TSB) and incubated for 24 hours at 35°C. Individually, every bacterial colony was spread on TSA plates for 48 hours' incubation period at 35°C. Individual organism colonies of each microbe, one by one and in separate test tubes, were shifted to TSB (10 ml) and incubated for 18–24 hours at 35 °C. These cultures were ready for the antimicrobial activities.

Antibacterial assay

A solitary colony of each culture was taken and shifted to TSB for overnight incubation. Hundred milliliter of melted Muller Hinton Agar was mixed with three milliliters of each culture at about 45 °C and dispensed onto agar plate surfaces. Each individual sample extract (80 µL) having 500 mg/mL concentration was poured on top of sterilized Ø 8mm filter paper disc two times with air drying in between. Every disc was kept on the tested microbial culture plates for 24 hours at 35 °C in incubator. Zone of inhibition along with disc diameter was calculated and recorded (Bauer et al., 1959; Bauer et al., 1966; Lennette, et al., 1991 and Jirawan et al., 2006).

Statistical measurement

Every parameter analysis was noted in triplicate. Results were expressed in terms of mean ± SD (n = 3), with the help of computer program SPSS.

Results and Discussion

Preliminary phytochemicals' screening was conducted to identify various secondary metabolites in M. communis leaves, stem and flower extracts. The results are expressed in table 1. The leaves have more phytochemicals as compared to the flowers and stems.

The antibacterial results are shown in table 2. The antibacterial activity of extracts is in the order methanol > n-hexane > aqueous > ethanol extract. While the plant parts' activity is in the order leaves > flower > stem.

Several herbs have been used to treat different diseases in Pakistan. Our elder generations trust the treatment of ailments by the traditional medicine because it gives a positive impact. On the other hand, increasing resistance of pathogenic bacteria and continuous changes in the resistance form of these microorganisms have led to the major challenges in the use of common antibiotic drugs which require search for new compounds with anti-bacterial properties.

The plants which possessed terpenoids, steroids, flavonoids, saponins, alkaloids and tannins have anti-diarrhoea activity (Havagiray et al., 2004; Brijesh et al., 2009; Yadav et al., 2007). Flavonoids have anti-diarrhoea potential because of their capability to shut down hydroelectrolytic secretions and intestine motion as they are recognized to be distorted in diarrhea (Venkatesan et al., 2005). In intestines, mucosa tannins break down proteins which produce tannates, and ultimately slow down secretion. Similar functions are also carried out by tannins like decreasing the inward current of intracellular Ca, or Ca pumping system activation which leads to the muscle relaxation (Belemtougri et al., 2006).

Myrtus communis leaves contained phenolic compounds (Hayder et al., 2008) and their antimicrobial activities were due to the presence of these compounds (Cakir et al., 2004). Polyphenol compounds attack cell membranes, cell wall, and imbalance porosity, and discharge cell ingredients; disturb membrane activities like nutrient uptake, enzyme functions, and electron transport. These compounds could have many targets which might result in the growth inhibition of microbes (Amensour et al., 2010).

Antimicrobial activity of M. communis leaves may be possible because of the tannin which synthesizes H bonds with proteins; which results in breaking down its shape, and thus stops protein formation (Khder 2008). Antibacterial phenomena of tannins are; direct effects on bacterial metabolism in the course of reduction of iron deprivation or oxidative phosphorylation or indirect scarcity of food required for bacterial growth, and slowing down the bacterial extracellular enzymes (Scalbert, 1991).

The Gram-negative microbes are more resistant to plants' extracts as compared to Gram-positive bacteria. It is due to the cell wall hydrophilic shape. The general cell wall of Gram-positive bacteria is composed of polysaccharides lipoprotein which prevents accumulation of its constituents in the cell membrane, and prevents the entrance of hydrophobic oil of the bacteria (Reynolds 1996; Bajpai et al., 2008; Bezic et al., 2003). By explaining this phenomenon, a Gram-positive bacterium is more susceptible to the extract than Gram-negative bacteria. The findings (Ali et al., 2013) revealed that M. communis leaves' extract had MIC of S. aureus (0.2 mg/ml) and E. coli (8 mg/ml) and V. cholera (2 mg/ml). But the M. communis leaves' extract results in differences in our study may be due to the differences in the extracted essential oil in the concentration, extraction values, geographical localities of plants, even used concentrations and tested microbes in the present study.

Conclusion

The current research work concluded that the methanolic extract of leaves possess the highest phytochemicals and antibacterial potential as compared to the other extracts. Since leaves of M. communis are still in practice as a conventional herbal system, possessing various bioactive compounds which offer a systematic base for additional basic healthy cure systems. Finally, we can conclude from this study that the active compounds of leaves, stems and flowers' extracts are a potential source against bacteria causing

diarrhea. Additional investigations are therefore, required to rectify their safety, efficacy and further evaluations.

Acknowledgment
The laboratory assistance of Mr. Faqir Hussain (Senior Technician) of Food Technology Center of PCSIR Peshawar Pakistan is gratefully acknowledged.

Conflict of Interest
The researchers of this study have declared no conflicts of interest by any means.

References:

1. Ali T, Amir S, Samira J, Fatemeh N. 2013. Antibacterial Effect of Myrtus Communis Hydro-Alcoholic Extract on Pathogenic Bacteria. Zahedan J Res Med Sci. 15(6): 19-24.
2. Amensour M, Bouhdid S, Fernandez-Lopez J, Idaomar M, Senhaji NS, Abrini J. 2010. Antibacterial Activity of Extracts of Myrtus communis Against Food-Borne Pathogenic and Spoilage Bacteria. Int J Food. 13 (6):1215–1224.
3. Bajpai VK, Rahman A, Kang SC. 2008. Chemical composition and inhibitory parameters of essential oil and extracts of Nandina domestica thumb to control food-borne pathogenic and spoilage bacteria. Int J Food Morobiol. 125(2): 117-122.
4. Bauer AW, Kirby WMM, Sherries JC, Turckp M.1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.
5. Bauer AW, Perry DM, Kirby WMM. 1959. Single disc antibiotic sensitivity testing of Staphylococci. Arch. Int. Med. 104: 208-216.
6. Belemtougri RG, Constantin B, Cognard C, Raymond G, Sawadogo L. 2006. Effects of two medicinal plants Psidium guajava L. (Myrtaceae) and Diospyros mespiliformis L. (Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture. J Zhejiang Univ Science B. 7(1):56–63.
7. Bezic N, Skocibusic M, Dinkic V and Radonic A. 2003. Composition and antimicrobial activity of Achilleaclavennae L. essential oil. Phytother Res. 17(9): 1073- 1040.
8. Brijesh S, Daswani P, Tetali P, Antia N, Birdi T. 2009. Studies on the antidiarrhoeal activity of Aegle marmelos unripe fruit: Validating its traditional usage. BMC Complement Altern Med. 9(47):1–12.
9. Cakir A, Kordali S, Zengin H, Izumi S, Hirata T. 2004. Composition and antifungal activity of essential oils isolated from Hypericum hyssopifolium and Hypericum heterophyllum. Flavour Fragrance J. 19(1): 62-68.
10. Dawson W. 2005. Herbal medicine and the EU directive, J R Coll Physicians Edinb. 35: 25-27.
11. De Smet PAGM, 2002. Herbal remedies. New Engl J Med. 347:2046–2056.
12. Dubey, NK, Kumar R and Tripathi P. 2004. Global promotion of herbal medicines: India's opportunity. Current Science. 86: 37-41.
13. Elif AE., Gulden G., Ayse E. 2014. Anti-candidal activities of some Myrtus Communis L. extracts obtained using accelerated solvent extraction (ASE). Journal of Applied Biology and Biotechnology. 2 (05): 012-014.
14. Farhana AR, Laizuman N, Mahmuda H, Monirul I. 2009. Antibacterial, Cytotoxic and Antioxidant Activity of Crude Extract of Marsilea Quadrifolia. European Journal of Scientific Research. 33(1): 123-129.
15. Fransworth NR. 1993. Ethnopharmacology and future drug development: the North American experience. Journal of Ethnopharmacol. 38: 45-152.
16. Harborne JB. Phytochemical methods - a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall; 1984, p. 4-16.
17. Havagiray R, Ramesh C, Sadhna K. 2004. Study of antidiarrhoeal activity of Calotropis gigantea r.b.r. in experimental animals. J Pharm Pharm Sci.7:70–75.
18. Haydar H, Mehmet MO, Derya A, Ahmet U. 2012. Biochemical compositional and technological characterizations of black and white myrtle (Myrtus communis L.) fruits. J Food Sci Technol. 49(1):82–88.

19. Hayder N, Bouhlel I, Skandrani I, Kadri M, Steiman R, Guiraud P, Mariotte AM, Ghedira K, Dijoux-Franca MG, Chekir-Ghedira L. 2008. In vitro antioxidant and antigenotoxic potentials of myricetin-3-ogalactoside and myricetin-3-o-rhamnoside from Myrtus communis: Modulation of expression of genes involved in cell defense system using cDNA microarray. ToxicolIn Vitro. 22(3): 567-581.
20. Houghton, PJ. 1995. The role of plants in traditional medicine and current therapy. Journal of Altern and Complement Med.1: 131-143.
21. Jirawan O, Tomoko S, Piyawan G, Griangsak E. 2006. Antimicrobial properties and action of galangal (Alpiniagalanga Linn.) on Staphylococcus aureus. LWT; 39: 1214–1220.
22. Khder AK. 2008. Effect of Allium sativum and Myrtus communis on the elimination of antibiotic resistance and swarming of Proteus mirabilis. Jordan J Biol Sci. 1 (3):124–8.
23. Lennette, TH, Barilows A, Hausler, WJJR, and Shadony HJ. 1991. Manual of clinical microbiology. 5th Edition. Washington, DC: American Society for Microbiology.
24. Reynolds JEF. Martindale the extra harmacopoeia. 31st Edition. London: Royal Pharmaceutical Society of Great Britain; 1996: 1681-1682.
25. Scalbert A. 1991. Antimicrobial properties of tannins. Phytochemistry. 30: 3875–3883.
26. Trease G, Evans SM. Pharmacognosy. 15th ed. London: Bailer Tindal; 2002, p. 23-67.
27. Venkatesan N, Thiyagarajan V, Narayanan S, Arul A, Raja S, Kumar SGV, Rajarajan T, Perianayagam JB. 2005. Antidiarrheal potential of Asparagus racemous wild root extracts in laboratoire animals. J Pharm Pharmaceut Sc. 8(1):39–45.
28. Vickers A and Zollman C. 1999. ABC of complementary medicine: herbal medicine. BMJ. 319:1050 - 1053.
29. WHO, (1985). 5th Programme Report, Programme for control of diarrhoeal diseases, Geneva. WHO Bulletin, 63: 557-772.
30. Yadav AK, Tangpu V. 2007. Antidiarrheal activity of Lithocarpus dealbata and Urena lobata extracts: therapeutic implications. Pharm Biol. 45(3):223–229.

Table 1. Phytochemicals analysis of M. communis

Phytochemicals	Extracts											
	Aqueous			Methanol			n-Hexane			Ethanol		
	L	S	F	L	S	F	L	S	F	L	S	F
Carbohydrate	++	+	+	+++	+	+	+++	+	+	+	+	-
Tannins	++	-	+	+++	+	+	+++	+	+	+	-	-
Saponins	-	-	-	+++	-	+	+++	-	-	+	-	-
Alkaloids	+	-	-	++	+	+	++	-	+	+	+	+
Glycosides	+	+	+	++	+	+	++	+	+	+	+	-
Phenols	++	+	+	+++	++	++	+++	+	++	++	+	+
Flavonoids	++	+	+	+++	++	++	+++	+	++	++	+	+
Terpenoids	-	-	+	+++	-	+	+	+	+	+	-	-

Key: +++ = Highly present, ++ = Moderately present, + = Low present, - = Absent, L= Leaves, S=Stem, F= Flower

Table 2. Antibacterial Activity of M. communis Extracts

Bacteria	Parts	Zone of Inhibition Diameter (mm)				C^{+T}	C^{d}
		Extracts					
		Aqueous	Methanol	n-Hexane	Ethanol		
Bacillus subtilis	L	27±1	32±2	29±2	24±1	30±2	00

	S	18±1	23±2	21±2	19±1		
	F	22±1	26±2	20±2	18±1		
Bacillus cereus	L	26±1	30±1	28±1	22±1	29±2	00
	S	17±1	22±1	21±1	16±1		
	F	20±1	24±1	21±1	17±1		
Staphylococcus aureus	L	25±1	31±1	30±1	23±0	31±1	00
	S	18±1	24±1	21±1	17±0		
	F	21±1	25±1	23±1	15±0		
Escherichia coli	L	15±0	23±0	21±1	15±1	20±1	00
	S	14±0	20±0	19±1	15±1		
	F	14±0	20±0	18±1	14±1		
Pseudomonas aeruginosa	L	00	22±1	19±1	00	20±1	00
	S	00	17±1	00	00		
	F	00	17±1	00	00		
Salmonella Typhi	L	18±1	23±1	20±1	17±1	21±1	00
	S	00	18±1	15±1	13±1		
	F	17±1	20±1	19±1	15±1		
Shigella sonnei	L	17±1	21±0	18±1	10±2	22±1	00
	S	12±1	15±0	13±1	00		
	F	14±1	18±0	15±1	00		
Shigella flexneri	L	16±1	23±1	19±0	15±2	19±1	00
	S	14±1	17±1	00	00		
	F	13±1	17±1	15±0	11±2		
Shigella dysenteriae	L	00	20±1	17±1	00	18±1	00
	S	00	15±1	14±1	00		
	F	00	14±1	13±1	00		
Vibrio cholerae	L	13±0	19±2	18±1	11±2	24±2	00
	S	12±0	16±2	00	09±2		
	F	13±0	16±2	00	10±2		