Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 8
Open Access

Potential Antimicrobial and Anti-Ulcer Properties of a Solvent Extracted Active Constituent from Amaranthaceae Family

Rahul Vasant Jadhav*, Dr. Praveen Sharma
Faculty of Pharmacy, Oriental University, Indore, Madhya Pradesh, India
*Corresponding Author: **Email address**: rahuljadhav671@gmail.com

Cite this paper as: Rahul Vasant Jadhav, Dr. Praveen Sharma (2024), Potential Antimicrobial and Anti-Ulcer Properties of a Solvent Extracted Active Constituent from Amaranthaceae Family. *Frontiers in Health Informatics*, *13(8) 4620-4631*

ABSTRACT:
Background and Aim: Medicinal herbs can be used as antacids in a number of Ayurvedic treatments and are a rich source of antibacterial properties. Many artificial antacids are frequently used to treat hyperacidity. Nevertheless, drugs made from plants may work well for creating new antacids. Therefore, the purpose of the current study was to evaluate the acid-neutralizing and antibacterial properties of an extract of native plants belonging to the Amaranthaceae family. Methodology: Fordtran's model titration method was used to ascertain the neutralization capability in vitro. The broth dilution method was used to determine the minimum inhibitory concentration (MIC), and the disc diffusion method was used to evaluate antibacterial activity. Result: The result of this study showed, 100 mg of Methanolic extract of Achyranthes aspera (MEAA) and Water extract of Achyranthes aspera (WEAA) had a stronger acid neutralizing effect. At a concentration of 250 g/disc, the highest activity of plant extract in MEAA was observed at 26.00 mm diameter ZOI against P. vulgaris, followed by 25.00 mm diameter ZOI against E. coli. Next was Methanolic extract of Amaranthus spinosus (MEAS), which has a 21.00 mm ZOI against E. coli. Conclusion: Out of all the extracts examined, MEAS and MEAA extracts exhibited the highest capacity to neutralize acids. In vitro, MEAS extract showed the highest antibacterial activity against both gram-positive and gram-negative bacteria.

KEYWORDS: Amaranthaceae, Methanolic extract, Antioxidant, Antiulcer, Extraction

INTRODUCTION:

Numerous illnesses caused by human pathogenic microorganisms are treated with antibiotics. Since the bacteria causing the illnesses are growing more resistant and changing into strains that are resistant to several treatments, it has been shown that the developed medications are not particularly effective in treating infections. To tackle these diseases in humans, new antibiotics must be introduced. Although synthetic derivatives or synthetic antibiotics are effective, using them can be risky, and the results are not satisfactory because of the bacteria's capacity to build resistance. Overall, the negative responses and side effects brought on by wide Spectrum antibiotics are important to consider [1]. These circumstances necessitate the investigation, introduction, and use of plant-derived compounds or phytomedicines with antibacterial activity carried out using biological and clinical experiments.

With origins spanning more than 3,000 years, Ayurvedic medicine has grown to become one of India's most important traditional medical systems. Many studies conducted recently have demonstrated that plants, which are essential to Ayurvedic treatments, generate a wide range of bioactive chemicals with important industrial applications. Due to their promising therapeutic properties, these natural chemicals have gained growing interest in the pharmaceutical and medical industries and have proven to be a viable source for the development of numerous drugs [2]. Bioactive chemicals, which are abundant in medicinal and aromatic plants, are essential to their interactions with the environment. Numerous secondary metabolites with strong antibacterial qualities, including terpenes, alkaloids, flavonoids, and phenolic acids, are produced by these plants [3]. Therefore, these plants' antibacterial qualities also make them useful in traditional medicine, where they are applied to cure illnesses and advance well-being [4].

Although the stomach's normal production of acid is essential for the digestion of food, too much of it can result in acidity. Eructation, dyspepsia, and heartburn are typical signs of acidity. When used orally, antacids neutralize excess stomach acid to reduce symptoms.
Acid neutralizing capacity (ANC) is the most commonly used metric to assess an antacid's strength. The amount of milliequivalents (mEq) of 1N hydrochloric acid that may be neutralized to a pH of 3.5 in 15 minutes with a single dose of an antacid product is known as the acid-neutralizing capacity (ANC) [5,6]. Although several synthetic antacids are used to treat hyperacidity, there is increasing interest in investigating plant-based natural alternatives.

Particularly, plants in the Amaranthaceae family exhibit promise as sources for novel natural antacids. In order to assess the acid-neutralizing ability and antibacterial potential of solvent extracts from native plants in the Amaranthaceae family, the current study was carried out.

MATERIALS AND METHODS:

Capacity to neutralize acid:

Selected Extracts

The following are the extracts with abbreviations used for the study: PEAS:

Petroleum ether extracts of Amaranthus spinosus; CEAS: Chloroform extracts of Amaranthus spinosus; MEAS: Methanol extracts of Amaranthus spinosus; WEAS: Water extracts of Amaranthus spinosus; PEAA: Petroleum ether extracts of Achyranthes aspera; CEAA: Chloroform extracts of Achyranthes aspera; MEAA: Methanol extracts of Achyranthes aspera; WEAA: Water extracts of Achyranthes aspera.

Artificial gastric acid preparation:

Dissolve 2 g of sodium chloride and 3.2 mg of pepsin in 500 ml of clean water. Add 7.0ml hydrochloric acid and enough water to prepare 1000ml artificial stomach acid solution. The pH of stomach acid is adjusted to 1.20. In 500 millilitres of clean water, dissolve 3.2 milligrams of pepsin and 2 grams of sodium chloride. To make a 1000 ml artificial stomach acid solution, combine 7.0 ml hydrochloric acid with adequate water. Stomach acid's pH is brought down to 1.20.

Preparation of plant extract:

The extracts' antacid properties were assessed at 100 mg/mL dosages. The volume of the test fluid was 90 millilitres. First, stock solutions of 100 mg/mL of the extracts were made using deionized water and 100% ethanol.

pH Determination of the extracts:

At a temperature between 25°C and 37°C, the pH of 90 milliliters of each test sample was determined. The pH values of water and sodium bicarbonate (SB) were also computed for comparison.

Neutralizing capacity determination on artificial gastric acid:

To check for neutralization, 100 ml of juice was mixed with 90 ml of freshly made test solutions, 90 ml of water, and 90 ml of active control SB. The pH was measured at 1.2.

Determination of the neutralization capacity in vitro using the titration method of Fordtran's model:

Freshly prepared 90 millilitres of each test solution were placed in a 250 ml beaker and heated to 37 degrees Celsius. 136 air bubbles per minute were used for aeration. A magnetic stirrer that was continuously rotated at 30 rpm was used to mimic stomach movements. Use artificial fruit juice to titrate the test sample until it reaches pH 3. 0.063096 (mmol/ml) × V (mL) is the formula used to determine total H+ (mmol) consumption [7].

In-vitro antimicrobial activity:

Test Microorganisms and Growth Media:

Based on their clinical and pharmacological significance, the following bacteria and yeast strains were selected: Salmonella typhi (MTCC 98), Micrococcus luteus (MTCC 106), Staphylococcus aureus (MTCC 96), Bacillus cereus (MTCC 7278), Proteus vulgaris (MTCC 8427), Pseudomonas aeruginosa (MTCC 1688), Bacillus niger (MTCC 282), Candida albicans (MTCC 227), and Aspergillus clavatus (MTCC 1323). On nutritional agar and potato dextrose agar (PDA) medium, respectively, bacterial and fungal cultures were cultivated for 24 hours at 37°C before being chilled to 4°C. While

yeasts and molds were grown in Sabouraud dextrose agar and PDA medium at 28°C, respectively, bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (bacteria were maintained on nutrient agar slants at 4°C and cultured in nutrient broth at 37°C). At 4°C, the stock cultures were maintained.

Sample preparation:

The bactericidal activity of the extracts was determined at concentrations of 5.00–250.00 µg/ml. After being measured, the selected extracts were added to DMSO to create a stock solution with a 250.00 µg/ml strength. Using the serial dilution procedure, the stock solution was used to establish concentrations of 5.00 µg/ml, 25.00 µg/ml, 50.00 µg/ml, 100 µg/ml, and 250 µg/mL for different concentrations that are produced in this way.

Determination of Zone of Inhibition:

The antibacterial activity was assessed using the disc diffusion method [8]. Using a sterile swab, each inoculum suspension (108 CFU/mL) was evenly distributed across the nutritional agar surface. 6 mm-diameter discs were autoclaved for 15 minutes at 1210C prior to being charged with ampicillin (20 µg/ml) as a positive control and different concentrations of extracts. After three to five minutes of drying, the impregnated discs were distributed using flamed forceps across the infected plates' surface. To ensure that every disc touched the nutritional agar surface completely, it was firmly pushed downward. The discs were sufficiently separated from one another and stopped moving as soon as they made contact with the agar surface. Following that, marking took place on plates that were incubated for 24 hours at 37 degrees Celsius for both bacteria and fungus. The zone of inhibition (ZOI), which is measured in millimeters, represented the degree of bacterial or fungal growth inhibition surrounding each disc.

Minimum inhibitory concentration (MIC):

With a few adjustments, the broth dilution method was used to determine the minimum inhibitory concentration [9]. For both primary and secondary screening, serial dilutions were prepared. 1000 µg/ml, 500 µg/ml, and 250 µg/ml extract concentrations were employed during initial screening. A second round of dilution against all bacteria was applied to the active extracts found in this initial screening. Concentrations of 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, and 6.250 µg/ml were achieved by diluting the extracts.

This antibiotic-free control tube was subcultured right away (before to inoculation) by one loopful, which was then evenly disseminated on a quarter of a plate with enough medium for the test organism's growth. It was then incubated for the entire night at 37 degrees Celsius. The tubes are then left in an incubator for the night. Drug concentration accuracy is verified using the control organism's MIC. The term "MIC" refers to the lowest concentration at which the organism cannot grow. The growth of the original inoculums in the control tube prior to incubation was compared.

RESULTS AND DISCUSSION:

Acid Neutralizing Effect: on artificial gastric acids

The pH values of the extracts—PEAS, CEAS, MEAS, WEAS, PEAA, CEAA, MEAA, and WEAA—were determined after adding 100 mg (in 90 ml) of the test solution extracts to 100 ml of artificial gastric juice (pH 1.2). The pH values of sodium

bicarbonate (SB) and water solutions were also measured for comparison, and they were 1.37 ± 0.00 and 1.75 ± 0.00, respectively. (Table 1)

Sr. No.	Drug / Extract	pH value
1	Water	1.37±0.00
2	Standard (SB)	1.75±0.00*
3	PEAS 100 mg	1.39±0.02
4	CEAS 100 mg	1.41±0.01*
5	MEAS 100 mg	1.61±0.01*
6	WEAS 100 mg	1.58±0.02*
7	PEAA 100 mg	1.42±0.03
8	CEAA 100 mg	1.49±0.01
9	MEAA 100 mg	1.64±0.01*
10	WEAA 100 mg	1.58±0.02*

Table 1: Determination of the neutralizing capacity on artificial gastric acid

Data are presented as mean ± SEM (n = 6) P* <0.05 when compared with water, SB: Sodium Bicarbonate.

Acid Neutralizing Effect: In-vitro

For water, sodium bicarbonate, PEAS, CEAS, MEAS, WEAS, PEAA, CEAA, MEAA, and WEAA extract solutions, the volumes of artificial gastric juices needed to titrate to pH 3.0 were 1.4±0.03, 33.15±0.45*, 6.56±0.09*, 7.32±0.08*, 10.23±0.05*, 9.56±0.03*, 5.36±0.21*, 8.57±0.07, 9.98±0.06*, and 9.12±0.05*, respectively. 00.08±0.00, 2.65±0.04*, 0.6±0.00*, 0.6±0.00*, 0.5±0.00*, 0.5±0.00*, 0.5±0.00*, 0.5±0.00*, 0.5±0.00*, and 0.6±0.00* mmol were the amounts of H+ that were consumed, respectively. (Table 2). All of the extracts were significantly more neutralizing than water, but less so than sodium bicarbonate. Every extract exhibited potent antacid properties.

Sr. No.	Drug / Extract	Consumed volume of artificial gastric	Mmol of H+

		c juice (ml)	
1	Water	1.4±0.03	0.08±0.00
2	Standard (SB)	33.15±0.45*	2.65±0.04*
3	PEAS 100 mg	6.56±0.09*	0.6±0.00*
4	CEAS 100 mg	7.32±0.08*	0.6±0.00*
5	MEA	10.23±0.05*	0.5±0.00*

	S 10 0 m g		
6	W E A S 10 0 m g	9.56± 0.03*	0.6± 0.00 *
7	PE A A 10 0 m g	5.36± 0.21*	0.5± 0.00 *
8	C E A A 10 0 m g	8.57± 0.07	0.5± 0.00 *
9	M E	9.98± 0.06*	0.5± 0.00

	A A 10 0 m g		*
1 0	W E A A 10 0 m g	9.12± 0.05*	0.6± 0.00 *

P*<0.05 when compared with water, Data are presented as mean ± SEM (n = 6). SB: Sodium Bicarbonate

"The neutralizing effect on artificial gastric juice may be used as a gauge of when antacids start to work because the pH is directly measured when the sample solution is added to a fixed volume of the simulated stomach acid. It is an important factor to take into account when assessing antacid potential since an effective antacid must be able to react with acids quickly" [10,11]. Conversely, MEAA 100 mg and WEAA 100 mg demonstrated a more potent neutralizing effect. These results are consistent with those obtained from the extracts' capacity to neutralize acids.

In-Vitro Antimicrobial Activity:

Zone of inhibition:

At a concentration of 250 µg/disc, the MEAA (Methanol Extract of Achyranthes aspera) plant extract demonstrated the highest antimicrobial activity against Proteus vulgaris, exhibiting a 26.00 mm zone of inhibition. This was followed by a 25.00 mm zone of inhibition against Escherichia coli. Furthermore, at a dose of 250 µg/disc, MEAA demonstrated a 22.00 mm zone of inhibition against Pseudomonas aeruginosa and a comparable 22.00 mm zone against Salmonella typhi.

At the same concentration of 250 µg/disc, the MEAS (Methanol Extract of Amaranthus spinosus) extract also demonstrated strong antimicrobial activity, exhibiting a 21.00 mm zone of inhibition against Escherichia coli, a 24.00 mm zone against Pseudomonas aeruginosa, a 22.00 mm zone against Proteus vulgaris, and a 23.00 mm zone of inhibition against Salmonella typhi.

At a concentration of 250 g/disc, the maximal activity of the plant extract in WEAA was found to be 20.00 mm in diameter of the zone of inhibition against P.

aeruginosa and E. coli, followed by 19.00 mm in diameter of the zone of inhibition against S. typhi. At a dose of 250 g/disc, WEAS was found to have a zone of inhibition against S. typhi with a diameter of 21.00 mm, 19.00 mm against P. aeruginosa and E. coli, and 15.00 mm against P. vulgaris.

At a concentration of 250 µg/disc, the MEAA (Methanol Extract of Achyranthes aspera) showed strong antibacterial activity, according to the study. This was similar to the standard antibiotics ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin, which also tested at 250 µg/disc. The zones of inhibition for the plant extracts and the common antibiotics against the tested Gram-negative bacteria are graphically compared in Figure 1, which illustrates the significant efficacy of MEAA in preventing the growth of Gram-negative organisms.

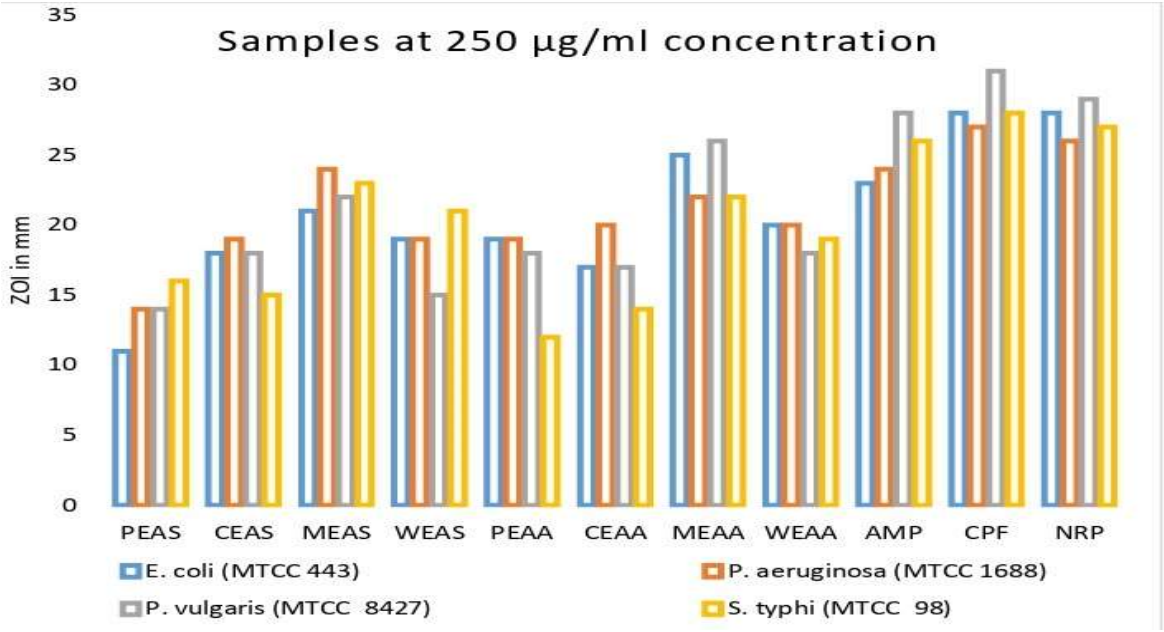

Figure 1: Graphical presentation of Zone of inhibition of extracts and standard antibiotics against Gram negative organism

At a concentration of 250 µg/disc, the different extracts—PEAS, CEAS, MEAS, WEAS, PEAA, CEAA, MEAA, and WEAA—showed zones of inhibition against Bacillus subtilis with diameters of 15 mm, 18 mm, 20 mm, 17 mm, 19 mm, 17 mm, 19 mm, and 17 mm, respectively, against Gram-positive organisms. At the same dose, the extracts MEAS and MEAA had significant efficacy against Staphylococcus aureus, with zones of inhibition measuring 21.00 mm and 20.00 mm, respectively.

Furthermore, at 250 µg/disc, MEAS and WEAS each showed a zone of inhibition against Micrococcus luteus with a diameter of 20.00 mm. At the same concentration, MEAS and MEAA showed zones of inhibition of 19.00 mm and 20.00 mm, respectively, for Bacillus cereus.

MEAS and MEAA demonstrated strong antibacterial effectiveness against the tested Gram-positive pathogens when compared to conventional antibiotics. Figure 2 shows the graphical representation of the zones of inhibition for these extracts against

Gram-positive bacteria in combination with common antibiotics.

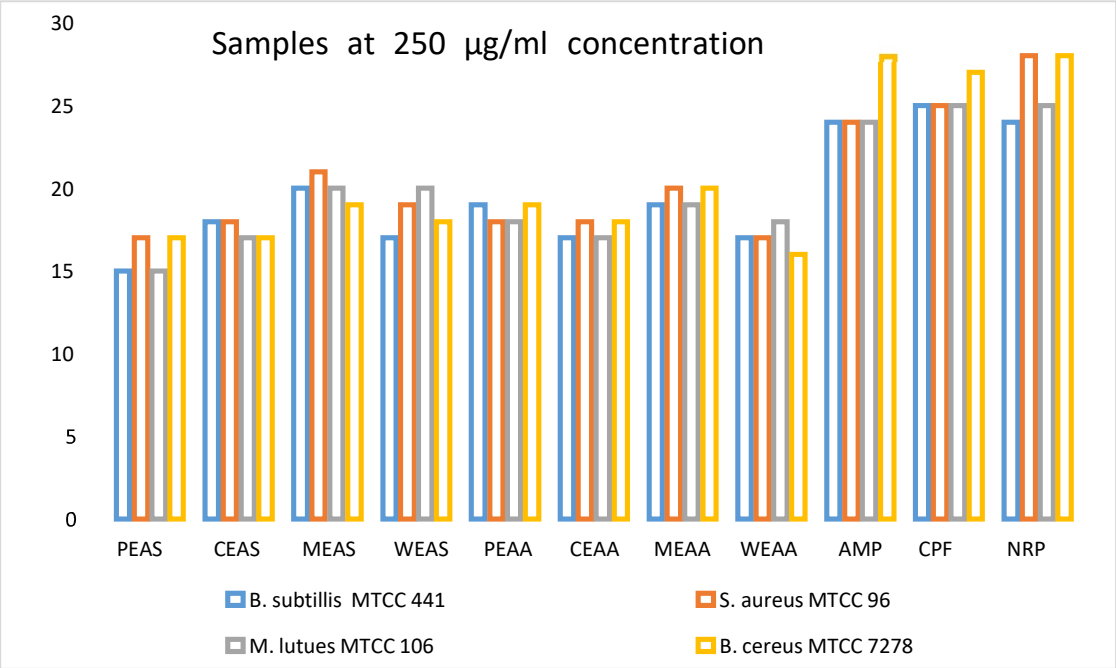

Figure 2: Graphical presentation of Zone of inhibition of extracts and standard antibiotics against Gram-positive organism

Minimum Inhibitory Concentration (MIC) Measurement:

A modified broth dilution approach was used to find the minimum inhibitory concentration (MIC) [12]. For the species under investigation, the MIC values varied between 50 and 500 µg/ml. In particular, MIC values ranged from 50 to 500 µg/ml for tested Gram-positive bacteria and from 100 to 500 µg/ml for tested Gram-negative bacteria. According to these findings, the plant extract is more efficient against Gram-positive bacteria than Gram-negative bacteria, even at lower doses. The higher sensitivity of Gram-positive bacteria to the plant extract is highlighted by this difference in MIC values, suggesting that the extract may be a more effective antibacterial agent against these organisms.

Figure 3 shows the MIC of several solvent extracts against both Gram-positive and Gram-negative organisms graphically.

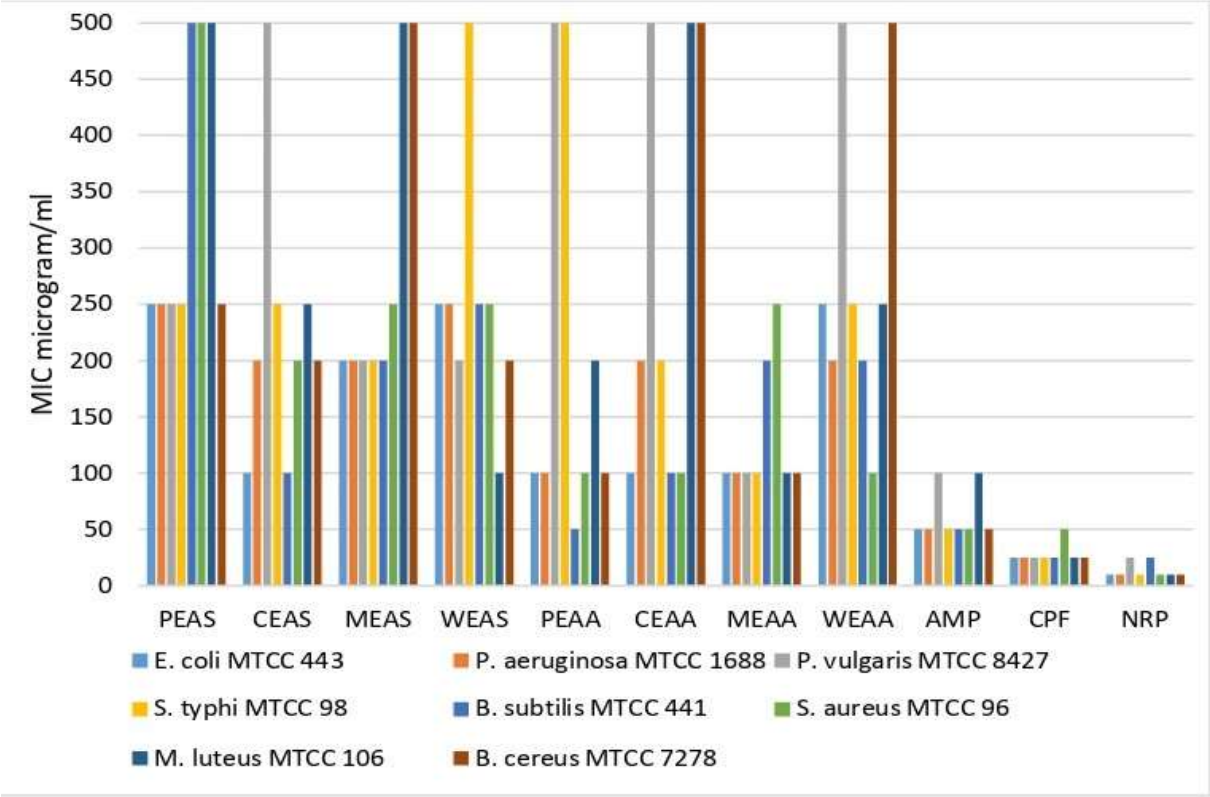

Figure 3: Graphical presentation of Minimum Inhibitory Concentration of different solvent extracts against Gram-positive and Gram-negative micro-organisms

CONCLUSION:

When compared to the other plant extracts assessed in the study, the MEAS and MEAA extracts had a noticeably higher acid-neutralizing capacity (ANC). This implies that these particular extracts are better at neutralizing stomach acid, which could make them useful natural antacids. The MEAS extract was the most effective of the evaluated samples in terms of antibacterial activity. Against both Gram-positive and Gram-negative bacteria, it demonstrated the strongest antibacterial activity in vitro. This suggests that MESS is a good option for additional research as a natural antibacterial agent due to its broad-spectrum antimicrobial action. MEAS's exceptional ability to stop bacterial growth indicates that it may be utilized to treat infections brought on by a variety of bacterial diseases.

ACKNOWLEDGEMENT:

The author would like to acknowledge the Faculty of Pharmacy, Oriental University, Indore for providing the necessary facilities for the research.

FUNDING:
Nil

CONFLICT OF INTEREST:
Declared None

REFERENCES:

1. Shaik G, Sujatha N, Mehar SK. Medicinal plants as source of antibacterial agents to counter Klebsiella pneumonia. J Applied Pharm Sci. 2014, 4(1), 135-147.
2. Aidi Wannes W, Mhamdi B, Sriti J, Ben Jemia M, Ouchikh O, Hamdaoui G, Kchouk ME, Marzouk B.. Antioxidant activities of the essential oils and methanol extracts from myrtle (Myrtus communis var. italica L.) leaf, stem and flower. Food Chem Toxicol. 2010, 48, 1362-70.
3. Chopra RN, Nayer SL, Chopra IC. Glossary of Indian Medicinal Plants, 3rd Edn. New Delhi: Council of Scientific and Industrial Research. 1992, pp.7-246.
4. Kane JH, Finlay AC, Sobin BA. Antimicrobial agents from natural sources. Ann. NY Acad. Sci. 1950, 53, 226–228.
5. Rao GCS, Sahoo NK, Parhi R, Panigrahi N. Study on the effectiveness of branded and generic antacid suspension forms. J Pharm Res. 2011; 4(3):612-613.
6. Shah K, Parmar S, Virpura M. Physiochemical, phytochemical investigation and in vitro acid neutralizing capacity of an antacid polyherbal formulation. Ind J Pharma Res Develop. 2011; 3(5):64-69.
7. Fordtran JS, Morawski SG, Richardson CT. In vivo and in vitro evaluation of liquid antacids. N Engl J Med. 1973; 288:923-928.
8. Balouiri M, Sadiki M, Ibnsouda SK. Methods for in vitro evaluating antimicrobial activity: A review. J Pharm Anal. 2016;6(2):71-79.
9. Kowalska-Krochmal B, Dudek-Wicher R. The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. Pathogens. 2021;10(2):165.
10. Grinshpan D.D.; Nevar T.N.; Savitskaya T.A.; Boiko A.V.; Kapralov N.V.; Sholomitskaya I.A. Pharm. Chem. J. 2008, 42(7), 400-404.
11. Lirazan M, Cua S. J, Alvarez M. R. In Vitro Antacid Screening of the Aqueous and Ethanolic Leaf Extracts of Triticum Aestivum (Linn.) and Hordeum Vulgare (Linn.). Orient J Chem 2018;34(1).
12. Carson CF, Hammer KA, Riley TV. Broth micro-dilution method for determination of susceptibility of Escherichia coli and Staphylococcus aureus to the essential oil of Malaleuca alterifolia (Tea tree oil). Microbios 1995, 82, 181-185.