Bulletin of Pure and Applied Sciences
Zoology (Animal Science), Vol.43B, No.2S,
July-December 2024: P. 887-895

Print version ISSN 0970-0765
Online version ISSN 2320-3188

Original Research Article
Content Available online at: www.bpasjournals.com

ANTIMICROBIAL AND ACID NEUTRALIZING POTENTIAL OF SELECTED SOLVENT EXTRACT FROM AMARANTHACEAE FAMILY

Rahul Vasant Jadhav*, Dr. Praveen Sharma
Faculty of Pharmacy, Oriental University

Corresponding Author
Rahul Vasant Jadhav
Research Scholar,
Faculty of Pharmacy, Oriental University,
Indore, Madhya Pradesh 453555
Email: rahuljadhav671@gmail.com

ABSTRACT

Background and Aim: Medicinal herbs are a rich source of antibacterial agents and can also be utilised as antacids in a variety of Ayurvedic preparations. Hyperacidity is often treated with various artificial antacids. Despite this, plant-derived medications could be effective in developing novel antacids. Thus, the current work was performed to assess the extract of indigenous plants of the Amaranthaceae family for acid-neutralising capacity and antibacterial potential.

Method: The neutralisation capacity was determined in vitro using Fordtran's model titration approach. Antibacterial activity was assessed employing the disc diffusion method, and the minimum inhibitory concentration (MIC) was established using the broth dilution method.

Results: Alternatively, MEAA 100 mg and WEAA 100 mg showed a more powerful neutralizing effect. The largest activity of plant extract in MEAA was found to be at 26.00 mm diameter of ZOI against P. vulgaris followed by 25.00 mm diameter ZOI against E.coli at a concentration of 250 g/disc. Then, there was MEAS which had a ZOI of 21.00 mm against E.coli.

Conclusion: MEAS and MEAA extracts had the maximum acid-neutralizing potential of all the extracts tested. MEAS extract demonstrated the strongest antimicrobial activity against both gram-negative and positive bacteria in vitro.

Keywords: *Amaranthus spinosus, Achyranthes aspera,* antimicrobial activity, acid neutralization, heartburn.

INTRODUCTION

Antibiotics are used to manage a variety of diseases produced by human pathogenic bacteria. It has been discovered that the created drugs are not very successful in treating infections since the microorganisms responsible for the infections are becoming resistant and evolving into multidrug-resistant strains. The introduction of new antibiotics is required to combat these human infections. Synthetic derivatives or synthetic antibiotics are effective yet

dangerous to employ, and the outcomes are unsatisfactory owing to the bacteria' resistance development capability. Altogether, the adverse reactions and side effects generated by broad-spectrum antibiotics should not be overlooked [1]. As a result of these factors, plant-derived substances or phytomedicines with antibacterial activity must be investigated, introduced, and implemented through clinical and biological experiments.

Ayurvedic medicine has its roots in India, dating back over 3,000 years, and has since become one of the foundational traditional healing systems of country. In current periods, extensive research has shown that plants, which are central to Ayurvedic practices, produce a diverse array of bioactive compounds with significant industrial relevance. These natural compounds have proven to be a valuable source for developing various medications, offering promising therapeutic effects that have garnered increasing attention in the medical and pharmaceutical fields [2]. Medicinal and Aromatic plants are rich in bioactive compounds that play a crucial role in their interactions with the surrounding environment. These plants produce a wide range of secondary metabolites, such as alkaloids, flavonoids, terpenes, and phenolic acids, which have potent antimicrobial properties [3]. Thus, the antimicrobial properties of these plants also make them valuable in traditional medicine, where they are used to treat infections and promote health [4].

The stomach naturally secretes acid, which is necessary for food digestion, but too much of it can cause acidity. Common acidity symptoms include heartburn, dyspepsia, and eructation. Antacids relieve symptoms by neutralising excess stomach acid when used orally. The most frequent measurement of an antacid's potency is acid neutralising capacity (ANC). The acid-neutralizing capacity (ANC) refers to the number of milliequivalents (mEq) of 1N hydrochloric acid that can be neutralized to a pH of 3.5 within 15 minutes by a single dose of an antacid product [5,6]. While various synthetic antacids are widely used to manage hyperacidity, there is growing interest in exploring natural alternatives derived from plants. Plants from the Amaranthaceae family, in particular, show potential as sources for the development of new natural antacids. Therefore, the present study was conducted to evaluate the acid-neutralizing capacity and antimicrobial potential of solvent extracts from indigenous plants of the Amaranthaceae family.

MATERIALS AND METHODS

Acid neutralizing capacity:-

Extracts Selected

The extract with abbreviation taken for study are as follows

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.

Preparation of plant extracts:

The antacid action of extracts was evaluated at doses of 100 mg/mL. The test solution volume was 90 millilitres. Stock solutions of the extracts (100 mg/mL) were first produced in 100% ethanol and deionised water.

pH Determination of the extracts:

The pH value of 90 ml of each test sample was measured at a temperature of 25°C to 37°C. For comparison purposes, the pH values of sodium bicarbonate (SB) and water were also calculated.

Neutralizing capacity determination on artificial gastric acid:

Freshly prepared 90 ml of each test solution, water (90 ml) and active control SB (90 ml) were added to juice (100 ml) at pH 1.2. Measure the pH to examine the neutralization of the juice.

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

A 250 ml beaker was filled with freshly made ninety millilitres of each test solution and warmed to 37 degrees Celsius. Aeration was performed at 136 air bubbles per minute. To simulate stomach movements, a magnetic stirrer was spun constantly at 30 rpm. Titrate the test sample with artificial fruit juice to pH 3. Total H+ (mmol) consumption is calculated as 0.063096 (mmol/ml) × V (mL) [7].

In-vitro antimicrobial activity:-

Test Microorganisms and Growth Media:

"Escherichia coli (MTCC 443), *Proteus vulgaris* (MTCC 8427)*, Bacillus cereus* (MTCC 7278), *Pseudomonas aeruginosa* (MTCC 1688), *Bacillus subtilis* (MTCC 441), *Salmonella typhi* (MTCC 98), *Micrococcus luteus* (MTCC 106), *Staphylococcus aureus* (MTCC 96), and fungal strains *Aspergillus niger* (MTCC 282), *Candida albicans* (MTCC 227), *Aspergillus clavatus* (MTCC 1323), were chosen based on their clinical and pharmacological importance. Bacterial and fungal cultures were cultured for 24 hours at 37°C on nutritional agar and potato dextrose agar (PDA) medium, respectively, and then refrigerated at 4°C. Bacterial strains were cultivated in Mueller-Hinton agar (MHA) plates at 37°C (bacteria were cultured in nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas yeasts and moulds were grown in Sabouraud dextrose agar and PDA medium at 28°C, respectively". The stock cultures were kept at 4°C.

Sample preparation:

At doses of 5.00-250.00 µg/ml, the antibacterial activity of the extracts was ascertained. The chosen extracts were measured and put into DMSO, to make a stock solution with a strength of 250.00 µg/ml. Concentrations of 5.00 µg/ml, 25.00 µg/ml, 50.00 µg/ml, 100µg/ml and 250 µg/mL were established from the stock solution through serial dilution method using the stock solution for various concentrations that are derived in this manner.

ZOI Determination:

The disc diffusion method was used to evaluate the antibacterial activity [8]. Each inoculum suspension (108 CFU/mL) was spread uniformly on the entire nutritional agar surface with a sterile swab. Discs of 6 mm in diameter were autoclaved at 1210C for 15 minutes before charging with a positive control (ampicillin, 20 µg/ml) and various concentrations of

extracts. Drying impregnated discs took 3-5 minutes and then they were scattered over the surface of the inoculated plates using flamed forceps. Each disc was strongly forced down to make complete contact with the nutrient agar surface. The discs were adequately spaced out and became immobile when they touched the agar surface. Subsequently, marking occurred on plates which were left to incubate for a period of twenty four hours at thirty seven degrees centigrade for both bacteria as well as fungi. The level of bacterial or fungal growth inhibition around each disc was expressed as zone of inhibition (ZOI) measured in millimeters.

MIC Determination:

The minimum inhibitory concentration was obtained using the broth dilution method, with some modifications [9]. Serial dilutions were made for primary and secondary screening. During primary screening, extract concentrations of 1000 µg/ml, 500 µg/ml, and 250 µg/ml were used. The active extracts discovered in this primary screening were subjected to a second round of dilution against all bacteria. Dilutions of the extracts were made to have concentrations of 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml and 6.250 µg/ml. This control tube having no antibiotic was immediately subculture (before inoculation) by one loopful of spreading it uniformly over a quarter of a plate with enough medium for the growth of the test organism and then incubated at 37 degrees Celsius overnight. Subsequently, the tubes are put in an incubator overnight. The MIC for the control organism is used to confirm that drug concentrations are correct. The lowest concentration that allows no growth of the organism is referred to as MIC. Growth in the control tube before incubation representing original inoculums was compared.

RESULTS AND DISCUSSION

Acid Neutralization Capacity:

Determination of the neutralizing effects on artificial gastric acids:

"When 100 mg (in 90 ml) of the test solution extracts were added to 100 ml of artificial gastric juice (pH 1.2), the pH values of the extracts—PEAS, CEAS, MEAS, WEAS, PEAA, CEAA, MEAA, and WEAA—were measured. For comparison, the pH values of water and sodium bicarbonate (SB) solutions were also determined, which were found to be 1.37 ± 0.00 and 1.75 ± 0.00, respectively." (Table 1).

Table: Determination of the neutralizing capacity on artificial gastric acid

Sr. No.	Drug/Extracts	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*

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

Determination of the neutralization capacity in vitro:

"The consumed volumes of artificial gastric juices to titrate to pH 3.0 for water, Sodium bicarbonate, PEAS, CEAS, MEAS, WEAS, PEAA, CEAA, MEAA and WEAA extracts solutions were found to be 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. The consumed H+ were 00.08±0.00, 2.65±0.04*, 0.6±0.00*, 0.6±0.00*, 0.5±0.00*, 0.6±0.00*, 0.5±0.00*, 0.5±0.00*, 0.5±0.00* and 0.6±0.00*mmol, respectively" (Table 2). All of the extracts had lower neutralising capabilities than sodium bicarbonate but much higher than water. All of the extracts had strong antacid efficacy.

Table 2: Consumed volume of artificial gastric juice

Sr. No.	Drug/Extracts	Consumed volume of artificial gastric juice (ml)	Mmol of H+
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	MEAS 100 mg	10.23±0.05*	0.5±0.00*
6	WEAS 100 mg	9.56±0.03*	0.6±0.00*
7	PEAA 100 mg	5.36±0.21*	0.5±0.00*
8	CEAA 100 mg	8.57±0.07	0.5±0.00*
9	MEAA 100 mg	9.98±0.06*	0.5±0.00*
10	WEAA 100 mg	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

"Because the resultant pH is directly evaluated upon addition of the sample solution to a fixed volume of the simulated gastric acid, the neutralising impact on artificial gastric juice may be utilised as a measure of the beginning of action of antacids. It is a crucial feature to consider when evaluating antacid potential since one requirement of a good antacid is its ability to react quickly with acids" [10,11]. MEAA 100 mg and WEAA 100 mg, on the other hand, had a stronger neutralising impact. These findings are congruent with those found in the extracts' acid-neutralisation capabilities.

***In-Vitro* Antimicrobial Activity:**

Zone of inhibition:

In the study, the maximum antimicrobial activity of the MEAA (Methanol Extract of Amaranthaceae A) plant extract was observed against *Proteus vulgaris*, with a zone of inhibition measuring 26.00 mm in diameter, followed by a 25.00 mm zone of inhibition against *Escherichia coli* at a concentration of 250 µg/disc. Additionally, MEAA showed a 22.00 mm zone of inhibition against *Pseudomonas aeruginosa* and a similar 22.00 mm zone against *Salmonella typhi*, both at a dose of 250 µg/disc.

Similarly, the MEAS (Methanol Extract of Amaranthaceae S) extract exhibited significant antimicrobial activity, with 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*, all at the same concentration of 250 µg/disc.

In WEAA, the maximum activity of plant extract was determined to be 20.00 mm diameter of zone of inhibition against *E. coli* and *P. aeruginosa* followed by 19.00 mm diameter of zone of inhibition against *S. typhi* at a concentration of 250 g/disc. WEAS was discovered to have a 21.00 mm diameter zone of inhibition against *S. typhi***,** 19.00 mm against *P. aeruginosa* and *E. coli* at a concentration of 250 g/disc, 15.00 mm against *P. vulgaris* at a dose of 250 g/disc.

The study revealed that the MEAA (Methanol Extract of Amaranthaceae A) demonstrated significant antibacterial activity at a concentration of 250 µg/disc, comparable to the standard antibiotics ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin, each also tested at 250 µg/disc. The effectiveness of MEAA in inhibiting the growth of Gram-negative organisms was notable, and these results are visually represented in Figure 1, which provides a graphical comparison of the zones of inhibition for both the plant extracts and the standard antibiotics against the tested Gram-negative bacteria..

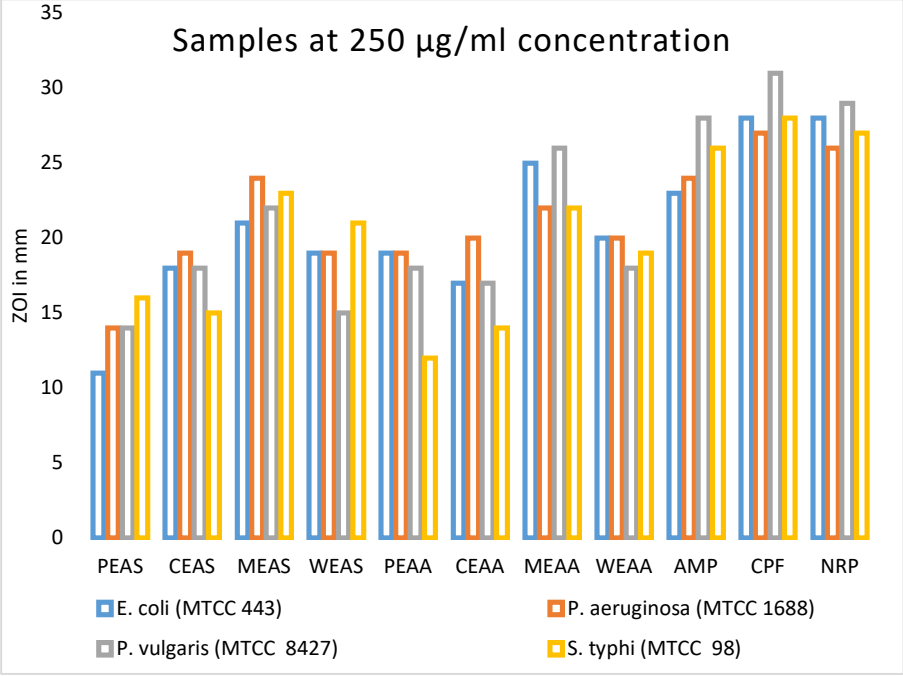

Figure 1: Graphical presentation of Zone of inhibition of extracts and standard antibiotics against Gram negative organism

"Against Gram-positive organisms, the various extracts—PEAS, CEAS, MEAS, WEAS, PEAA, CEAA, MEAA, and WEAA—exhibited zones of inhibition with diameters of 15 mm, 18 mm, 20 mm, 17 mm, 19 mm, 17 mm, 19 mm, and 17 mm, respectively, against *Bacillus subtilis* at a concentration of 250 µg/disc. The extracts MEAS and MEAA showed notable activity against *Staphylococcus aureus*, with zones of inhibition measuring 21.00 mm and 20.00 mm, respectively, at the same concentration.

Additionally, MEAS and WEAS each exhibited a 20.00 mm diameter zone of inhibition against *Micrococcus luteus* at 250 µg/disc. For *Bacillus cereus*, MEAS and MEAA demonstrated zones of inhibition of 19.00 mm and 20.00 mm, respectively, at the same concentration.

In comparison to standard antibiotics, MEAS and MEAA showed significant antibacterial activity against the Gram-positive organisms tested. The graphical presentation of the zones of inhibition for these extracts, along with standard antibiotics, against Gram-positive organisms is depicted in Figure 2.

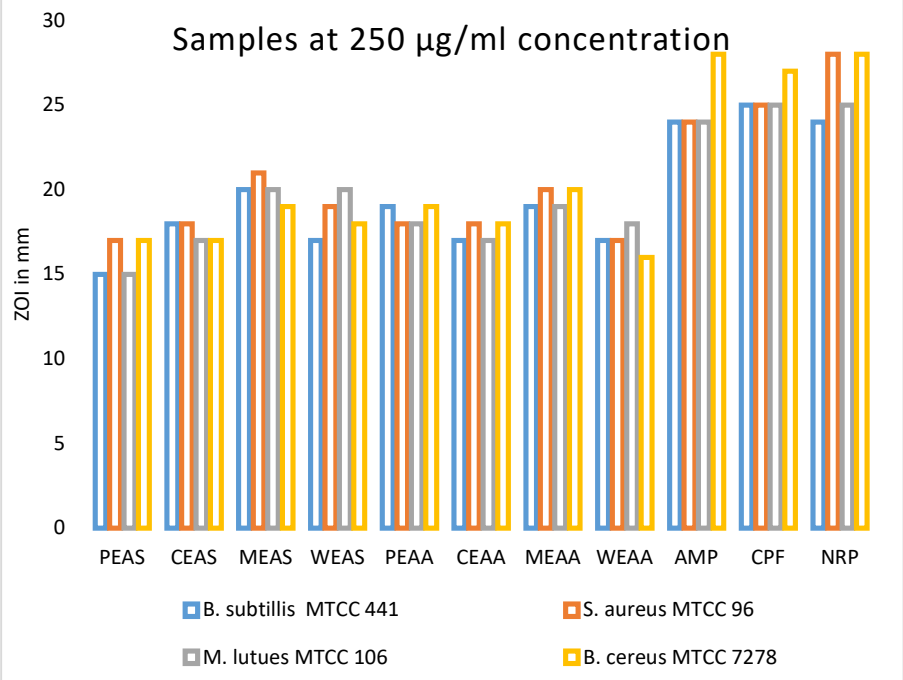

Figure 2: Graphical presentation of Zone of inhibition of extracts and standard antibiotics against Gram-positive organism

Minimum Inhibitory Concentration (MIC) Measurement:

The minimum inhibitory concentration (MIC) was determined using a modified broth dilution method [12]. The MIC values for the examined species ranged from 50 to 500 µg/ml. Specifically, the MIC values against the tested Gram-positive bacteria varied from 50 to 500 µg/ml, whereas the MIC values against the tested Gram-negative bacteria ranged from 100 to 500 µg/ml. These results suggest that the plant extract exhibits greater antibacterial effectiveness against Gram-positive bacteria at lower concentrations compared to Gram-negative bacteria. This difference in MIC values highlights the higher sensitivity of Gram-positive bacteria to the plant extract, indicating its potential as a more potent antibacterial agent against these organisms.

The Graphical presentation of MIC of different solvent extracts against Gram-positive

and Gram-negative organism is depicted in figure 3.

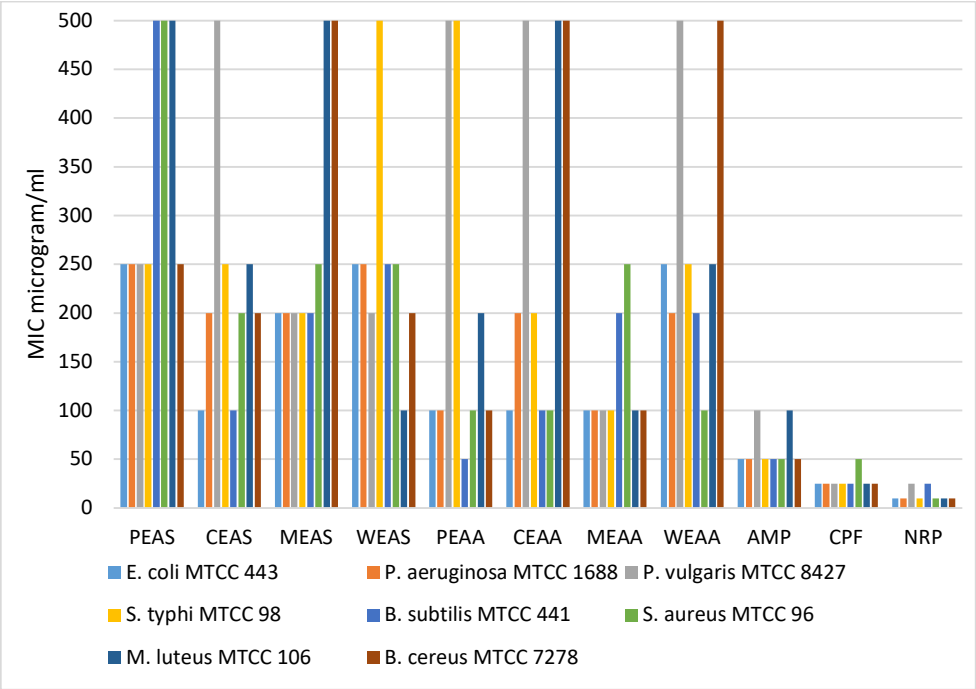

Figure 3: Graphical presentation of MIC of different solvent extracts against Gram-positive and Gram-negative organism

CONCLUSION

The acid-neutralizing capacity (ANC) of the tested plant extracts was notably higher in the MEAS and MEAA extracts compared to the other extracts evaluated in the study. This suggests that these specific extracts have a superior ability to neutralize stomach acid, making them potentially effective natural antacids.

In terms of antimicrobial activity, the MECT extract stood out as the most potent among the tested samples. It exhibited the highest in vitro antibacterial activity against both Gram-negative and Gram-positive bacteria. This indicates that MECT has a broad-spectrum antimicrobial effect, making it a promising candidate for further development as a natural antibacterial agent. The superior performance of MECT in inhibiting bacterial growth highlights its potential use in treating infections caused by a wide range of bacterial pathogens.

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.