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Research Article

ACID NEUTRALIZING CAPACITY AND ANTIMICROBIAL POTENTIAL OF SELECTED SOLVENT EXTRACT FROM VARIOUS INDIGENOUS PLANTS

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ABSTRACT

Medicinal plants have been representing a rich source of antimicrobial agent as well as can be used as antacid in various ayurvedic preparations. Hence the present study was undertaken to evaluate the antibacterial potential and antacid properties of selected six plant extracts. The plant extracts such as Methanolic extracts of *Cassia tora* (MECT), Methanolic extracts of *Pithecellobium dulce* (MEPD), Chloroform extracts of *Butea monosperma* (CEBM), Butanolic extracts of *Pongamia pinnata* (BEPP), Water extracts of *Tephrosia purpurea* (WETP), Methanolic extracts of *Mucuna pruriens* (MEMP) were selected for the study. The neutralization capacity *in vitro* was performed using the titration method of Fordtran's model. The antimicrobial activity was performed by using the disc diffusion method and minimum inhibitory concentration was determined by broth dilution method. The consumed volumes of artificial gastric juices to titrate to pH 3.0 for water, Sodium bicarbonate, MECT, MEPD, CEBM, BEPP, WETP and MEMP extracts solutions were found to be 1.3±0.02, 34.22±0.59*, 8.26±0.08*, 10.58±0.09*, 9.6±0.1*, 7.35±0.05, 9.36±0.09* and 9.88±0.06* respectively. The highest activity of plant extract has been shown in MECT and was found to be 25.00 mm diameter of zone of inhibition against *E. coli* at the concentration of 250 µg/disc followed by 24.00 mm diameter of zone of inhibition against *P. aeruginosa* at concentration of 250 µg/disc. Against Gram positive organism, the MECT and MEPD possess 21.00 mm and 20.00 mm diameter of zone of inhibition respectively against *M. Lutues* at concentration of 250µg/disc. The MIC values were found in the range of 50-500µg/ml against the tested organisms. The acid neutralizing capacity was found to be higher in MEPD and MEMP extracts among all the selected extract. The highest *in vitro* antimicrobial activity was found in MECT extract against gram-negative and gram-positive bacteria.

Keywords: *Cassia tora, Pithecellobium dulce, Butea monosperma, Pongamia pinnata, Tephrosia purpurea,Mucuna pruriens*, Antimicrobial, Antacid.

1. INTRODUCTION

Herbal formulated medicine and traditional healthcare practice are globally perceived as comparatively cheaper and more widely accessible to most rural and less-privileged populations around the world than synthetic drugs and orthodox medicine respectively [1]. Herbal medicine has a long history in treatment of several diseases [2]. Medicinal herbs are used to treat illness, maintain and promote health [3] and are the only available and inexpensive source of primary health care, especially in the absence of access to modern medical facilities. Various plant parts (leaves, stems, root and bark) are used for medicine preparation, because they contain biologically active ingredients, for treating mild or chronic ailments. Alkaloids, tannins, flavonoids, and phenolic compounds are the most important bioactive constituents of plant. Sometimes they are also added to foods of pregnant and nursing mothers for medicinal purposes [4].

Ayurvedic medicine is originated in India more than 3,000 years ago and remains one of the country's traditional health care systems. In recent decades, research has shown that plants produce a diverse range of bioactive molecules for industrial interest, making them a rich source of different types of medicines and have shown a promising effect in therapeutics [5]. Aromatic and medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment and inhibit bacterial or fungal growth [6]. Thus medicinal plants have been representing a rich source of antimicrobial agent [7].

The stomach normally secretes acid which has an essential role in the digestion of food, although excess production of this may result in acidity. Heartburn,

dyspepsia and eructation are common symptoms of acidity. Antacids provide a symptomatic relief from these symptoms by neutralizing the excess gastric acid upon oral administration. Acid neutralizing capacity (ANC) is the most commonly used measure to express potency of an antacid. Acid neutralizing capacity can be defined as the number of milli equivalents (mEq) of 1N hydrochloric acid that is brought to a pH of 3.5 in 15 min by a unit dose of an antacid preparation [8, 9]. Various known artificial antacids are commonly used to treat hyperacidity. Despite this, drugs obtained from the plant kingdom may serve as useful sources in the development of new natural antacids. Hence, the present study was carried out to evaluate thedifferent solvent extract of indigenous plant for Acid Neutralizing capacity and antimicrobial potential.

2. MATERIAL AND METHODS

2.1. Acid neutralization properties

2.1.1. Selected Extracts

MECT: Methanolic extracts of *cassia tora*
MEPD: Methanolic extracts of *Pithecellobium Dulce*
CEBM: Chloroform extracts of *Butea Monosperma*
BEPP: Butanolic extracts of *Pongamia Pinnata*
WETP: Water extracts of *Tephrosia Purpurea*
MEMP: Methanolic extracts of *Mucuna Pruriens*

2.1.2. Preparation of plant extracts

Evaluation of antacid activity of extracts was carried out using concentrations of 100 mg/mL. The volume of test solution was 90mL. Stock solutions of the extracts (100 mg/mL) were initially prepared in absolute ethanol and deionized water.

2.1.3. Preparation of artificial gastric acid

A 2g of NaCl and 3.2 mg of pepsin enzymes were dissolved in 500 ml distilled water. Hydrochloric acid (7.0 ml) and adequate water were added to make a 1000 ml solution of artificial gastric acid. The pH of the artificial gastric acid solution was adjusted to 1.20.

2.1.4. Determination of pH of the extracts

The pH of ninety milliliters of each test solution was determined at temperatures ranging from 25°C to 37°C. The pH values of the sodium bicarbonate (SB) and water was also determined for comparison.

2.1.5. Determination of the neutralizing effects on artificial gastric acid

Freshly prepared ninety milliliters of each test solution; water (90 ml) and the active control SB (90 ml) were added separately to the artificial gastric juice (100 ml) at pH 1.2. The pH values were determined to examine the neutralizing effects on artificial gastric juice.

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

Freshly prepared ninety milliliters of each test solution was placed in a 250 ml beaker and warmed to 37°C. Aeration was given at 136 air bubbles per minute. A magnetic stirrer was continuously run at 30 rpm to imitate the stomach movements. The test samples were titrated with artificial gastric juice to the end point of pH 3. The consumed volume (V) of the artificial gastric juice was measured. The total consumed H+ (mmol) was measured as 0.063096 (mmol/ml) × V (ml) [10].

2.2. *In-vitro* antimicrobial activity

2.2.1. Test Microorganisms and Growth Media

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

2.2.2. Sample preparation

Antimicrobial activity of the extracts was tested at various concentrations ranging from 5.00-250.00 µg/ml. The selected extracts were weighed and dissolved in DMSO to prepare stock solution of 250.00 µg/ml concentrations. The same stock solution has been utilized to get desired concentrations of 5.00µg/ml, 25.00 µg/ml, 50.00 µg/ml, 100.00 µg/ml and 250.00 µg/ml by the serial dilutions method.

2.2.3. Determination of zone of inhibition (ZOI)

The antimicrobial activity was performed by using the disc diffusion method [11, 12]. In the assay, each

inoculums suspension (10^8 CFU/mL) was spread evenly over the entire nutrient agar surface by sterile collection swab. Then, discs having of diameter 6 mm were sterilized at 121°C for 15 min and loaded with prepared positive control (ampicillin, 20µg/ml) and extract solutions at various concentrations. The impregnated discs were dried for 3-5 min and dispensed onto the surface of the inoculated plates with flamed forceps. Each disc was pressed down firmly to ensure complete contact with nutrient agar surface. The discs were placed suitably apart and not relocated once contacted with the agar surface. The plates were then labeled and incubated at 37°C for 24 hours for both bacteria and fungus. The results were measured and expressed in terms of zone of inhibition (ZI) of bacterial and fungal growth around each disc in millimeters.

2.2.4. Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was determined by broth dilution method with some modification [13, 14]. Serial dilutions were prepared in primary and secondary screening. In primary screening 1000 µg/ml, 500 µg/ml, and 250 µg/ml concentrations of the extracts were taken. The active extracts found in this primary screening were further tested in a second set of dilution against all microorganisms. The extract found active in primary screening were similarly diluted to obtain 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml, 6.250µg/ml concentrations. The control tube containing no antibiotic is immediately sub cultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 °C overnight. The tubes are then incubated overnight. The MIC of the control organism is read to check the accuracy of the drug concentrations. The lowest concentration inhibiting growth of the organism is recorded as the MIC. The amount of growth from the control tube before incubation (which represents the original inoculums) is compared.

3. RESULTS AND DISCUSSION

3.1. Determination of the neutralizing effects on artificial gastric acids

When test solution extracts 100 mg (90 ml) was added to 100 ml of the artificial gastric juice (pH 1.2), the pH values of MECT, MEPD, CEBM, BEPP, WETP and MEMP extracts were found to be 1.54±0.01*, 1.44±0.02*, 1.53±0.01*, 1.51±0.01, 1.45±0.03, 1.49 ±0.01*, respectively. The pH values of water and Sodium bicarbonate solutions were 1.39 ± 0.00 and 1.72 ± 0.00, respectively (Table 1).

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

S. No	Drug	pH value
1	Water	1.39±0.00
2	Standard (SB)	1.72±0.00*
3	MECT 100 mg	1.54±0.01*
4	MEPD 100 mg	1.44±0.02*
5	CEBM 100 mg	1.53±0.01*
6	BEPP 100 mg	1.51±0.01
7	WETP 100 mg	1.45±0.03
8	MEMP 100 mg	1.49±0.01*

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

3.2. Determination of the neutralization capacity *in vitro*

The consumed volumes of artificial gastric juices to titrate to pH 3.0 for water, Sodium bicarbonate, MECT, MEPD, CEBM, BEPP, WETP and MEMP extracts solutions were found to be 1.3±0.02, 34.22±0.59*, 8.26±0.08*, 10.58±0.09*, 9.6±0.1*, 7.35±0.05, 9.36 ±0.09* and 9.88±0.06* respectively. The consumed H+ were 0.07±0.00, 2.15±0.03*, 0.5± 0.00*, 0.6± 0.00*, 0.5±0.00*, 0.5±0.00, 0.6±0.00* and 0.6± 0.00* mmol, respectively (Table 2). The neutralization capacities of all the extracts were lesser than that of Sodium bicarbonate but significantly better than that of water. All the extract exhibited significant antacid potency.

Table 2: Consumed volume of artificial gastric juice

S. No	Drug	Consumed volume of artificial gastric juice (ml)	mmol of H+
1	Water	1.3±0.02	0.07±0.00
2	Standard (SB)	34.22±0.59*	2.15±0.03*
3	MECT 100 mg	8.26±0.08*	0.5±0.00*
4	MEPD 100 mg	10.58±0.09*	0.6±0.00*
5	CEBM 100 mg	9.6±0.1*	0.5±0.00*
6	BEPP 100 mg	7.35±0.05	0.5±0.00
7	WETP 100 mg	9.36±0.09*	0.6±0.00*
8	MEMP 100 mg	9.88±0.06*	0.6±0.00*

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

The neutralizing effect on artificial gastric juice can be used as a measure of the onset of action of antacids since in this case, the resulting pH is directly determined upon addition of the sample solution to a fixed volume of the artificial gastric acid. It is an important factor and must be taken into account when evaluating antacid potential since one criterion of an ideal antacid is that it must react rapidly with acids [15, 16]. The neutralizing effect, however, was higher for MEPD and MEMP. These observations are consistent with those observed in the acid neutralization capacities of the extracts.

3.3. *In-vitro* antimicrobial activity

The highest activity of plant extract has been shown in MECT and was found to be 25.00 mm diameter of zone of inhibition against *E.coli* at the concentration of 250 µg/disc followed by 24.00 mm diameter of zone of inhibition against *P.aeruginosa* at concentration of 250 µg/disc. In comparison to ampicillin, chloramphenicol, ciprofloxacin and norfloxacin at 250µg/disc as shown in table 3, the MECT possess significant antibacterial activity at 250µg/disc.

Against Gram positive organism, the MECT and MEPD possess 21.00 mm and 20.00 mm diameter of zone of inhibition respectively against *M. Lutues*at concentration of 250µg/disc. However, BEPP possess 20.00 mm diameter of zone of inhibition against *B. cereus*at concentration of 250µg/disc. In comparison to standard drugs, MECT, MEPD and BEPP represent significant antibacterial activity (Table 4).

Table 3: Zone of inhibition of extracts and standard antibiotics against Gram negative organism

SN	Extr. code	Zone of Inhibition (mm)																			
		E. coli (MTCC 443)					*P. aeruginosa* (MTCC 1688)					*P. vulgaris* (MTCC 8427)					*S. typhi* (MTCC 98)				
		5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
Various extracts																					
1	MECT	-	14	17	20	25	-	12	16	21	24	-	8	16	19	22	-	8	12	17	18
2	MEPD	-	12	13	15	18	-	9	13	17	19	-	9	12	16	18	-	11	13	17	21
3	CEBM	-	10	12	17	19	-	8	12	15	20	-	11	14	18	22	-	10	12	16	19
4	BEPP	-	14	15	17	19	-	14	15	17	19	-	12	11	14	15	-	14	17	18	21
5	WETP	-	12	14	15	19	-	12	14	15	19	-	13	15	18	26	-	12	15	20	22
6	MEMP	-	15	16	18	20	-	15	16	18	20	-	15	13	17	17	-	11	12	12	14
Standard Antibiotics																					
7	AMP	14	15	16	19	23	14	15	15	18	24	15	19	20	24	28	12	15	19	24	27
8	CMP	14	17	23	23	23	14	17	18	19	23	13	16	22	25	29	10	13	18	21	25
9	CPF	20	23	28	28	28	20	23	24	26	27	17	20	23	28	31	16	19	22	25	29
10	NRP	22	25	26	27	29	18	19	21	23	26	13	19	24	27	29	15	17	21	24	28

Standard antibiotics-AMP: Ampicillin; CMP: Chloramphenicol; CPF: Ciprofloxacin; NRF: Norfloxacin

Table 4: Zone of inhibition of extracts and standard antibiotics against Gram positive organism

SN	Extr. Code	Zone of Inhibition (mm)																			
		B. subtillis MTCC 441					*S. aureus* MTCC 96					*M. lutues* MTCC 106					*B. cereus* MTCC 7278				
		5	25	50	100	250	5	25	50	100	250	5	25	50	100	250	5	25	50	100	250
Various extracts																					
1	MECT	-	12	14	15	17	-	11	14	16	19	-	14	16	18	21	-	12	13	15	17
2	MEPD	-	12	13	17	18	-	11	14	15	18	-	12	15	17	20	-	11	12	16	17
3	CEBM	-	10	13	14	17	-	12	14	18	21	-	10	12	14	15	-	12	14	15	19
4	BEPP	-	11	13	15	16	-	11	14	16	19	-	15	16	17	18	-	12	15	16	20
5	WETP	-	11	14	16	19	-	12	14	16	21	-	12	14	16	18	-	12	14	15	19
6	MEMP	-	10	12	14	17	-	12	14	15	18	-	12	13	15	17	-	10	15	17	18
Standard Antibiotics																					
7	AMP	11	14	16	18	24	10	13	14	16	24	12	15	16	19	24	13	15	17	20	28
8	CMP	10	13	19	20	24	12	14	19	20	25	11	17	18	20	26	12	16	16	19	25
9	CPF	16	19	21	21	25	17	19	21	22	25	14	15	16	18	25	14	15	18	21	27
10	NRP	18	19	20	21	24	19	22	25	26	28	13	15	19	20	25	14	18	21	24	28

Standard antibiotics-AMP: Ampicillin; CMP: Chloramphenicol; CPF: Ciprofloxacin; NRF: Norfloxacin

The MIC values were found in the range of 50-500 µg/ mlagainst the tested organisms. The MIC values against the tested gram-positive bacteria rangedfrom 50 to 500µg/ml and against gram-negative bacteria from 100 to 500µg/ml. Antibacterial potency of plant extract-against these bacteria expressed in MIC indicated theplant extract is more effective against gram-positive atlower concentration than that against gram-negativebacteria (table 5).

Table 5: Minimum inhibitory concentration of extracts and standard antibiotics against Gram positive and Gram negative organism

		Minimal inhibition concentration (µg/ml)							
SN	Extract Code	*E. coli* MTCC 443	*P. aeruginosa* MTCC 1688	*P. vulgaris* MTCC 8427	*S. typhi* MTCC 98	*B. subtilis* MTCC 441	*S. aureus* MTCC 96	*M. luteus* MTCC 106	*B. cereus* MTCC 7278
		Various extracts							
1	MECT	250	250	250	250	500	500	500	250
2	MEPD	100	200	500	250	100	200	250	200
3	CEBM	200	200	200	200	200	250	500	500
4	BEPP	250	250	200	500	250	250	100	200
5	WETP	100	100	500	500	50	100	200	100
6	MEMP	100	200	500	200	100	100	500	500
		Standard Antibiotics							
7	AMP	100	100	100	100	200	250	100	100
8	CMP	50	50	100	50	50	50	100	50
9	CPF	25	25	25	25	25	50	25	25
10	NRF	10	10	25	10	25	10	10	10

Standard antibiotics-AMP: Ampicillin; CMP: Chloramphenicol; CPF: Ciprofloxacin; NRF: Norfloxacin

4. CONCLUSION

The acid neutralizing capacity was found to be higher in MEPD and MEMP extracts among all the selected extract. The highest in vitro antimicrobial activity was found in MECT extract against gram-negative and gram-positive bacteria.

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