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EVALUATION OF LETHAL ACTIVITY OF *PSIDIUM GUAJAVA* LINN. EXTRACTS ON BACTERIAL PATHOGENS CAUSING DIARRHEAL INFECTIONS

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ABSTRACT

Treatment of diarrheal infections is often challenged by development of drug resistance among the bacteria. The aim of this study was to investigate the antibacterial property of extracts of guava (*Psidium guajava* Linn.) leaves against diarrhea causing bacterial pathogens. Four extracts viz., ethanol, methanol, ethyl acetate and hot water extract of guava leaf were assayed for antibacterial activity against three selected bacterial pathogens along with four commercial antibiotics. The methanol extract exhibited substantial inhibitory activity (100 %) against both kinds of bacteria with a MIC of > 250 µg/ml in most cases. The ethanol extract demonstrated antibacterial activity ranging from 38 % to 65 % with the inhibition of clinical isolate of *E. coli* at concentrations > 500 µg/ml and both strains of *V. cholerae* at > 750 µg/ml respectively. The ethyl acetate and hot water extracts showed comparatively lesser response with MICs > 750 µg/ml in some cases. The commercial antibiotic Chloramphenicol exhibited absolute inhibitory action on *Salmonella sp.* and clinical isolate of *V. cholerae*. While Tetracycline and Gentamycin caused complete bactericidal action on both the strains of *E. coli* and *V. cholerae* respectively, the clinical isolate of *E. coli* was totally susceptible to Erythromycin. On the other hand significant percentages of resistances to these antibiotics ranging from 63 % to 88 % were also recorded. Six phytochemical compounds presumed to possess pharmacological properties were detected from methanol extract. The findings suggest that the methanol extract of guava leaf could serve as a potential source to explore promising drugs to control diarrheal infections.

Keywords: *Psidium guajava* Linn., methanol extract, diarrheal infections, antibacterial, MIC

INTRODUCTION

The world Health Organization defines diarrhea as having three or more liquid stools per day. In developing countries diarrheal infection is given much attention as it is implicated as a common cause of morbidity and death of susceptible individuals. Frequent outbreaks of diarrhea occur as a consequence of poor maintenance of sanitation and personal hygiene. Worldwide approximately 2.5 billion cases of diarrhea occurred in 2004 costing 1.5 million deaths among children under the age of five; greater than the half of these occurred in Africa and South Asia. The diarrhea still remains to be the second leading cause of infant mortality (16 %) after pneumonia (17 %)[1]. Traveler's diarrhea (TD) is the most common travel related illness. It can occur anywhere but the highest risk destinations are in most of Asia as well as the Middle East, Africa, Mexico and Central and South America[2].

Diarrhea, in most cases, occurs as a consequence of food borne infections, where the improperly and un-hygienically prepared food stuff acts as vehicle of transmission of pathogens. Although the infectious gastroenteritis and diarrhea could be caused by various microorganisms the predominant agents often reported are enteric bacteria. Bacterial enteric pathogens cause approximately 80 % of TD cases. The death toll due to diarrhea caused by enteric bacteria has been estimated to be 50,000 people per day in most of the developing countries[3,4]. The bacterial diarrhea is generally acute in nature and often followed by ingestion of contaminated food. Although the bacterium *campylobacter* is a common cause of diarrhea, the infections by *Salmonella*, *Shigella* and some strains of *Escherichia coli* are frequent in most part of the world[5].

In recent years the use of antibiotics for the prophylaxis of bacterial diarrhea has been critically reviewed. The issues related to the ineffectiveness of antibiotics against viral or parasitic agents and the emergence of resistance among bacterial pathogens as a consequence of their indiscriminate use have been debated[6]. In order to circumvent this challenge and to accomplish effective and safe treatment against bacterial agents of diarrhea, the traditional system of medicine could be considered. Since ancient times the natural system of medicine employing plants and natural products has been offering veritable source of drugs. Antibacterial compounds have been isolated from a large number of plant species throughout the world[7-9].

Psidium guajava Linn., commonly known as Guava, is an edible plant primarily belong to Central America and part of South America[10]. This tree belongs to Myrtaceae family and is called regionally as amarood (India), babayas (Philippines), goyavier (France) guabang (Palau), guava (USA), etc. By virtue of its phyto-constituents this plant is most sought-after for its excellent medicinal properties. Various parts of this plant contain important organic chemicals which include tannin, essential oil

containing the sesquiterpene hydrocarbons, caryophyllene, β-bisabolene, aromadendrene, β-selinene, nerolidiol, caryophyllene oxide, some triterpenoids and β-sitosterol, polyphenols, resin, calcium oxalate, leukocyanidins, sterols, and gallic acid. In addition, it possesses calcium, phosphorous, magnesium, potassium, sodium, oleanolic acid, vitamin C, vitamin A, iron, calcium and phosphorus. Pharmacological studies advocate the potential application of different parts of guava plant for treating various ailments of human. The fruit, bark, leaf and root have been extensively employed in alternative medicine owing to their antibacterial, hypoglycemic, anti-inflammatory, analgesic, antipyretic, spasmolytic, and CNS depressant activities[11]. Many researchers had reported admirable therapeutic properties of guava leaf. The boiled water extract of guava plant leaves and bark are used in medicinal preparations employed for treating upper respiratory tract infections, wounds, ulcer, toothache, and stomach-ache. In other studies, anti-hyperglycemic, antipyretic[12], and bio-antimutagenic[13] properties of guava leaf extract had been demonstrated. The biological activity of noteworthy attributable to the flower and leaf of the plant has been the antibiotic activity. The antimicrobial, antidiarrheal, antimycobacterial[14,15], antimalarial activities[16] conferred by the chemical constituents of leaves of *P. guajava* L. had been reported by many researchers.

The diarrheal infections are often associated with the loss of electrolytes and essential nutrients thereby weakening the health of affected individuals. The condition could worsen if it is caused by drug resisting pathogens. As the leaf extracts of *P. guajava* L. have been proved to be promising sources of antimicrobial substances, this study has been conducted to investigate the inhibitory property of leaves of guava plant against selected diarrhea causing bacterial pathogens and to compare their efficacy with that of commercial antibiotics.

MATERIALS AND METHODS

Processing of plant material

The leaves of *Psidium guajava* L. were collected from Mambakkam, a sub-urban area of Chennai, India during September 2013. A voucher specimen has been deposited at the Center for Advanced Studies in Botany, University of Madras, Chennai, India (no. VS130359). The leaves were cleaned using sterile water and dried in a shady, well ventilated, dust free area. Dried leaves were ground up to coarse powder and processed for extraction.

Preparation of solvent and aqueous extracts

Two kilograms of air-dried leaf powder were extracted with 70 % ethanol : H_2O (7 : 3, 12 L) in soxhlet apparatus at room temperature, evaporated in vacuum using rotary flash evaporator (45°C) to 1.5 L. It was further filtered to remove the precipitated chlorophyll, concentrated and defatted with petrol. The percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies. Similar procedure was followed for methanol, ethyl acetate and hot water in order to prepare respective extracts[17].

Determination of phytochemical constituents

A portion of methanol extract was subjected to preliminary screening for phytochemical constituents using the modified method of Pandey and Shweta[18]. Tests were performed to detect in the extract of tannins, alkaloids, saponins, phlobatannins, flavonoids, terpenoids, reducing sugar and poly phenols.

Assay of Antimicrobial activity

Bacteria studied

Test organisms such as such as *Escherichia coli, Salmonella sp.* and *Vibrio cholerae* were employed for the assay of antibacterial activity of extracts of guava leaf. Standard type strains of these bacteria were obtained from Microbial Type Culture Collection center, Chandigarh, India. Clinical isolates of these bacteria were obtained from Institute of Basic Medical Sciences, University of Madras, Chennai, India.

Antibiotics used

Commercially available antibiotic powders were obtained from Hi Media Laboratories, India and tested along with the plant extracts. The selected antibiotics used in this study were Chloramphenicol - CL (CMS218), Gentamicin Sulphate - GEN (CMS461), Tetracycline hydrochloride - TC (CMS461) and Erythromycin – EM (CMS528).

Testing of antibacterial activity

The plant extracts were screened for antibacterial property using the agar well diffusion method as described by Nair and Chanda[19] with minor modifications. The pure culture of test organisms were grown in nutrient broth, optical density was compared with a 0.5 tube on the McFarland scale (10^8 CFU/ml) and swabbed (0.2 ml each) onto Mueller Hinton Agar (MHA) plates. Using sterile cork borer four wells (6 mm diameter) were cut in the agar maintaining a distance of 30 mm between the wells. Each extract was mixed with DMSO so as to achieve a concentration of 10 mg/ml (stock solution) and was loaded in the wells in different amounts viz., 25 µl (250 µg/ml), 50 µl (500 µg/ml), 75 µl (750 µg/ml) and 100 µl (1000 µg/ml). An amount of 0.1 ml of sterile DMSO or solvent alone was introduced in a separate well to serve as blank test / control. The plates were then incubated at 37°C for 24 h and the antibacterial activity was determined by measurement of diameter of halos of inhibition (mm) around each well. Similar method was followed for testing the action of commercial antibiotics. All the assays were done in triplicates and each experiment was repeated three times and the average of zone of inhibition was taken for that specific organism. For the reading and interpretation of the results only clear halos of 10 mm or more and devoid of bacterial growth were considered as halos of inhibition.

Table 1: Antibacterial activity of *P. guajava* L. leaf extracts on type strain and clinical isolates of *E. coli*

Culture tested	Parameters Recorded	Ethanol (µg/ml)				Methanol (µg/ml)				Ethyl acetate (µg/ml)				Hot water (µg/ml)			
		250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
E. coli Type strain (MTCC 433)	Halo size (mm)	5.0	11.0	18.0	20.0	11.0	13.0	18.0	25.0	5.0	6.0	12.0	18.0	3.0	6.0	8.0	13.0
	Sensitivity	R	S	S	S	S	S	S	S	R	R	S	S	R	R	R	S
	P value	0.752	0.034	0.008	0.001	0.093	0.027	0.001	0.001	0.381	0.173	0.082	0.006	0.446	0.631	0.057	0.002
E. coli Clinical isolate	Halo size (mm)	5.0	9.0	15.0	22.0	11.0	14.0	19.0	27.0	5.0	6.0	10.0	20.0	5.0	6.0	9.0	15.0
	Sensitivity	R	R	S	S	S	S	S	S	R	R	S	S	R	R	R	S
	P value	0.412	0.045	0.005	0.001	0.003	0.00	0.003	0.001	0.132	0.451	0.003	0.002	0.483	0.915	0.091	0.004

Table 2: Susceptibility of type strain and clinical isolates of *E. coli* to commercial antibiotics

Culture tested	Parameters Recorded	Chloramphenicol -CL (µg/ml)				Gentamicin -GEN (µg/ml)				Tetracycline -TC (µg/ml)				Erythromycin –EM (µg/ml)			
		250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
E. coli Type strain (MTCC 433)	Halo size (mm)	2.0	5.0	8.0	9.0	9.0	12.0	19.0	26.0	11.0	14.0	17.0	21.0	5.0	10.0	12.0	17.0
	Sensitivity	R	R	R	R	R	S	S	S	S	S	S	S	R	S	S	S
	P value	0.325	0.051	0.076	0.083	0.008	0.005	0.003	0.004	0.314	0.169	0.083	0.006	0.632	0.052	0.013	0.007
E. coli Clinical isolate	Halo size (mm)	3.0	5.0	8.0	10.0	11.0	14.0	19.0	27.0	13.0	15.0	19.0	23.0	11.0	14.0	18.0	21.0
	Sensitivity	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S
	P value	0.445	0.082	0.091	0.023	0.005	0.009	0.004	0.001	0.126	0.416	0.003	0.002	0.097	0.073	0.008	0.002

Table 3: Antibacterial activity of *P. guajava* L. leaf extracts on type strain and clinical isolates of *Salmonella sp.*

Culture tested	Parameters Recorded	Ethanol (µg/ml)				Methanol (µg/ml)				Ethyl acetate (µg/ml)				Hot water (µg/ml)			
		250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Salmonella sp. Type strain (MTCC 1167)	Halo size (mm)	3.0	5.0	8.0	12.0	6.0	11.0	20.0	28.0	2.0	3.0	3.0	7.0	4.0	6.0	7.0	13.0
	Sensitivity	R	R	R	S	R	S	S	S	R	R	R	R	R	R	R	S
	P value	0.798	0.263	0.007	0.004	0.082	0.015	0.004	0.001	0.672	0.564	0.621	0.218	0.842	0.785	0.072	0.006
Salmonella sp. Clinical isolate	Halo size (mm)	5.0	8.0	10.0	14.0	10.0	13.0	22.0	30.0	3.0	5.0	8.0	11.0	6.0	6.0	10.0	13.0
	Sensitivity	R	R	S	S	S	S	S	S	R	R	R	S	R	R	S	S
	P value	0.675	0.022	0.006	0.004	0.057	0.014	0.002	0.001	0.864	0.589	0.212	0.009	0.075	0.076	0.007	0.005

Table 4: Susceptibility of type strain and clinical isolates of *Salmonella sp.* to commercial antibiotics

Culture tested	Parameters Recorded	Chloramphenicol -CL (µg/ml)				Gentamicin -GEN (µg/ml)				Tetracycline -TC (µg/ml)				Erythromycin –EM (µg/ml)			
		250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
Salmonella sp. Type strain (MTCC 1167)	Halo size (mm)	12.0	14.0	18.0	24.0	6.0	9.0	18.0	23.0	2.0	5.0	8.0	12.0	6.0	10.0	13.0	19.0
	Sensitivity	S	S	S	S	R	R	S	S	R	R	R	S	R	S	S	S
	P value	0.017	0.008	0.008	0.005	0.009	0.082	0.003	0.002	1.293	1.049	0.357	0.019	0.329	0.018	0.009	0.008
Salmonella sp. Clinical isolate	Halo size (mm)	14.0	18.0	22.0	30.0	10.0	13.0	18.0	28.0	5.0	9.0	12.0	13.0	9.0	12.0	18.0	19.0
	Sensitivity	S	S	S	S	R	S	S	S	R	R	S	S	R	S	S	S
	P value	0.009	0.005	0.003	0.001	0.009	0.007	0.004	0.001	0.152	0.492	0.091	0.015	0.086	0.009	0.006	0.004

Table 5: Antibacterial activity of *P. guajava* L. leaf extracts on type strain and clinical isolates of *Vibrio cholerae*

Culture tested	Parameters Recorded	Ethanol (µg/ml)				Methanol (µg/ml)				Ethyl acetate (µg/ml)				Hot water (µg/ml)			
		250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
V. cholerae Type strain (MTCC 1167)	Halo size (mm)	5.0	8.0	14.0	19.0	13.0	18.0	23.0	29.0	4.0	4.0	6.0	8.0	5.0	6.0	8.0	16.0
	Sensitivity	R	R	S	S	S	S	S	S	R	R	R	R	R	R	R	S
	P value	0.087	0.023	0.005	0.003	0.007	0.005	0.001	0.001	0.463	0.463	0.252	0.017	0.761	0.713	0.066	0.004
V. cholerae Clinical isolate	Halo size (mm)	6.0	8.0	19.0	22.0	14.0	16.0	24.0	30.0	3.0	6.0	7.0	9.0	6.0	8.0	12.0	16.0
	Sensitivity	R	R	S	S	S	S	S	S	R	R	R	R	R	R	S	S
	P value	0.066	0.021	0.003	0.001	0.009	0.007	0.001	0.001	0.346	0.212	0.049	0.018	0.071	0.066	0.005	0.002

Table 6: Susceptibility of type strain and clinical isolates of *Vibrio cholerae* to commercial antibiotics

Culture tested	Parameters Recorded	Chloramphenicol -CL (µg/ml)				Gentamicin -GEN (µg/ml)				Tetracycline -TC (µg/ml)				Erythromycin –EM (µg/ml)			
		250	500	750	1000	250	500	750	1000	250	500	750	1000	250	500	750	1000
V. cholerae Type strain (MTCC 1167)	Halo size (mm)	8.0	11.0	14.0	19.0	14.0	18.0	22.0	28.0	4.0	5.0	9.0	13.0	7.0	9.0	14.0	19.0
	Sensitivity	R	S	S	S	S	S	S	S	R	R	R	S	R	S	S	S
	P value	0.026	0.013	0.005	0.002	0.009	0.005	0.001	0.000	0.935	0.629	0.085	0.017	0.563	0.012	0.007	0.008
V. cholerae Clinical isolate	Halo size (mm)	10.0	12.0	19.0	24.0	12.0	18.0	25.0	29.0	6.0	9.0	11.0	13.0	5.0	10.0	17.0	21.0
	Sensitivity	S	S	S	S	S	S	S	S	R	R	S	S	R	S	S	S
	P value	0.013	0.009	0.002	0.001	0.009	0.007	0.001	0.000	0.163	0.428	0.095	0.019	0.362	0.010	0.004	0.003

Table 7: Phytochemical compounds detected from the leaves of *P. guajava* L.

S. No.	Phytochemical Tested	Results
1.	Tannins	Positive
2.	Saponins	Positive
3.	Alkaloids	Negative
4.	Phlobatannins	Positive
5.	Flavonoids	Positive
6.	Terpenoids	Positive
7.	Reducing sugar	Positive

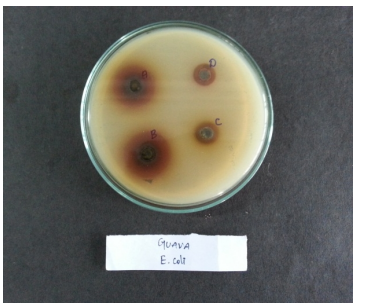

Figure 1: Inhibition of *Escherichia coli* by methanol extract of guava leaf

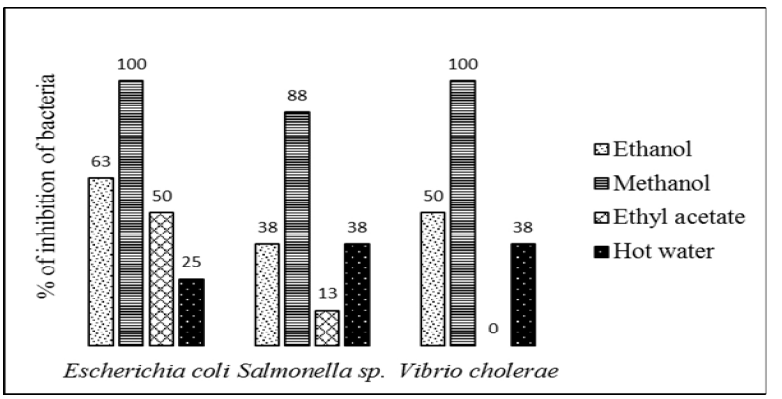

Figure 2: Inhibition (%) of diarrheagenic bacteria by different extracts of Guava leaf

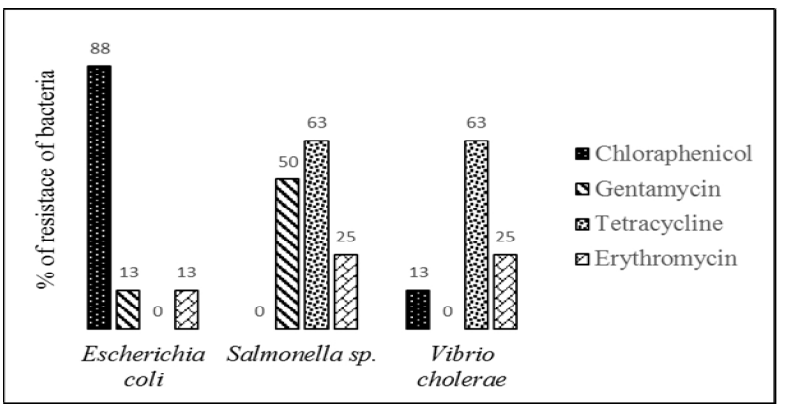

Figure 3: Resistance (%) exhibited by bacteria to different commercial antibiotics

The results were validated when no halo was observed around the well of the blank test, thus proving that the microbicidal action could be attributed to the compounds contained in the guava leaf extracts and not to the solvent.

Statistical Analysis

Analysis was done by comparing the mean diameter of the inhibition halos as a variable. Results were expressed as mean of zone of growth inhibition ± standard error (SE) of test organisms from several subjects. Statistical significance between the effects of drugs was determined using analysis of variance (if more than two) and paired Student's t test (between two groups) with $P > 0.005$ significance level.

RESULTS

Antibacterial activity of leaves of *Psidium guajava* L.

In the Tables 1 - 6 the sensitivity / resistance pattern of three test organisms to different concentrations of guava leaf extracts and the commercial antibiotics were noted.

The ethanol extract showed inhibitory activity against the type strain and clinical isolate of *E. coli* at concentrations > 500 µg/ml and > 750 µg/ml respectively. It could inhibit *Salmonella sp.* only at higher concentrations with the type strain showing more resistance (>

1000 µg/ml) than the clinical isolate (> 750 µg/ml) (Table 3). The bacteria *V. cholerae* exhibited moderate susceptibility to ethanol extract with the MIC of > 750 µg/ml in both cases (Table 5). The methanol extract demonstrated phenomenal lethal activity against all the three types of bacteria (Figure 1). Even at the lowest concentration (i.e., 250 µg/ml) it inhibited all the bacteria tested with the exception of type strain of *Salmonella sp.* which showed susceptibility at concentrations > 500 µg/ml (Figure 1; Tables 1, 3 and 5). There was a minimal antibacterial activity with respect to Ethyl acetate extract which could inhibit both types of *E. coli* and clinical isolate *Salmonella sp.* at concentrations > 750 µg/ml and > 1000 µg/ml respectively. It showed only negative effect on *V. cholerae* (Table 5). A least inhibitory activity was noted with hot water extract against all the bacteria tested. While it was effective only at > 1000 µg/ml on both types of *E. coli*, and type strains of *Salmonella sp.* (Tables 1 and 3) and *V. cholerae* (Table 5), a moderate activity (> 750 µg/ml) was recorded against the clinical isolates of *Salmonella sp.* and *V. cholerae* (Tables 3 and 5).

Susceptibility of bacteria to antibiotics

Assay of inhibitory action of commercial antibiotics indicated that Chloramphenicol was absolutely inhibitory against *Salmonella sp.* and *V. cholerae* excepting the type strain of *V. cholerae* (> 500 µg/ml) (Tables 4 and 6). Interestingly, there was a total and higher resistance (> 750 µg/ml) to this antibiotic respectively by the type strain and clinical isolate of *E. coli* (Table 2). Barring the type strain of *E. coli* (> 500 µg/ml), both types of *E. coli* and *V. cholerae* were totally susceptible to Gentamycin (Tables 2 and 6). There was a moderate to stronger action of this antibiotic on type strain (> 750 µg/ml) and clinical isolate (> 500 µg/ml) of *Salmonella sp.* respectively (Table 4). While there was a complete inhibitory action against *E. coli* by Tetracycline, its effect on other two bacteria was variable (Tables 2, 4 and 6). However, its effect was lower (> 1000 µg/ml) to moderate (> 750 µg/ml) respectively on type strain and clinical isolates of both *Salmonella sp.* and *V. cholerae*. Erythromycin showed comparatively a better inhibitory action on the bacteria tested. While the clinical isolate of *E. coli* was totally susceptible all the other bacteria were highly sensitive (> 500 µg/ml) to this antibiotic (Tables 2, 4 and 6).

Phytochemical constituents of leaves of *Psidium guajava* L.

The results of tests of phytochemical analysis of the leaf extract by using standard methods are summarized in Table 7. Out of the seven compounds tested, six predominant compounds were detected.

DISCUSSION

In the present study four different extracts viz., ethanol, methanol, ethyl acetate and hot water of leaves of *P. guajava* L. were investigated for their antibacterial activity against three commonly known agents of diarrhea. Infectious diarrhea in most cases occurs as a consequence of consumption of food or drink contaminated with food borne pathogens. It is characterized by alteration in normal bowel movement, abdominal discomfort and frequent stool dehydration along with other symptoms such as nausea, vomiting, cramps and fever[20]. Black *et al*.[21] had reviewed the most common agents of diarrhea in developing countries and reported that pandemic diseases are implicated with Rotavirus (15-25 %), entero-toxigenic *E. coli* (10-20 %), *Shigella* species (5-15 %) *Salmonella* species (1-5 %) *Campylobacter jejuni* (10-15 %) and other organisms (5-15 %) including those of entero-pathogenic *E. coli*, *Salmonella, Shigella* and *V. cholerae*. Although viruses constitute to be the main agents of diarrheal cases in developed countries. 50-60 % of cases in developing countries are attributed to bacterial agents[2].

The literatures indicate that the whole plant of *P. guajava* L. is admired for its therapeutic applications owing to the possession of various chemical constituents endowed with pharmacological properties. The leaves of this plant are given significant concern as they are widely used to treat various alimentary ailments in various parts of the world[22]. Traditionally the leaf extract is used in treatment of several ailments including inflammation, diabetes, hypertension, wounds, pain and fever[23]. Among the four extracts of guava leaf tested for antibacterial activity, the methanol extract has been proved to exhibit substantial activity with the 100 % lethal action on *E. coli* and *V. cholerae* and 88 % on *Salmonella sp.* (Figure 2). In most of the cases it could prevent the bacterial growth with the MIC level as low as 250 µg/ml. Fernandes Vieira *et al*.[5] had reported similar action of methanol extract on *E. coli*. The ethanol extract of the present study has demonstrated antibacterial activity ranging from 38 % (against *Salmonella sp.*) to 63 % (against *E. coli*) (Figure 2). The lowest MIC for this extract has been 500 µg/ml for *E. coli*. Comparatively lower antibacterial action has been recorded with ethyl acetate and hot water extracts. Although the ethyl acetate extract could show a moderate effect (50 %) on *E. coli*, it has been totally ineffective against *V. cholerae* (Figure 2). The hot water extract has been observed to be relatively inferior in action (25 %) against both types of *E. coli*. Interestingly, its effect on the clinical isolates of other two bacteria has been better than that of the type strains (MICs 750 µg/ml vs. 1000 µg/ml) (Tables 3 and 5).

The action of four commercial antibiotics tested against the three indicator organisms has been variable. A remarkable inhibitory action to the extent of 100 % has been recorded with Tetracycline (against *E. coli*), Chloramphenicol (against *Salmonella sp.*) and Gentamycin (against *V. cholerae*) (Tables 2, 4 and 6). Gnan and Demello[2] had reported similar finding where

the antibiotic Tetracycline showed stronger inhibitory effect on the test organisms. However, significant occurrence of antibiotic resistance ranging from 63 % (*Salmonella sp.* and *V. cholerae* against Tetracycline) to 88 % (*E. coli* against Chloramphenicol) (Figure 3) has also been noted in the present study.

The methanol extract employed in our study has been observed to be superior in its antibacterial action than the other three of its counterparts. Our finding is in agreement with the research work of Goncalves *et al*.[1], which demonstrated an overall lethal activity of methanol extract of guava leaf against gram negative bacteria including *E. coli* and *S. typhimurium*. The ethanol extract of the present study, although was variable in its action against the type strains of the test bacteria, its action against the clinical isolates has been satisfactory (MIC > 750 µg/ml) (Tables 1, 3 and 5). Concomitant with the reports of Goncalves and Neto[24], the ethyl acetate extract of our study showed a satisfactory antibacterial action on *E. coli* (MIC > 750 µg/ml) (Table 3). Similarly the hot water extract demonstrated satisfactory action (MIC > 750 µg/ml) on *Salmonella sp.* and *V. cholerae* (Tables 3 and 5). As the methanol extract of guava leaf was observed to be promising, the analysis of phytochemical contents has been carried out with it. The corresponding assays detected the occurrences of tannins, saponins, phlobatannins, flavonoids, terpenoids and reducing sugars. Similar findings had been achieved by Shihabudeen *et al*[25]. The antimicrobial property of guava leaf could be attributed to the phytochemicals contained in it. Components such as tannins, saponins, flavonoids and terpenes reportedly possess antibacterial and antioxidant activities[26]. The antibacterial activity of the methanol extract of guava leaf could be due to the presence of flavonoids, in particular the morin glycosides, quercetin glycosides and quercetin. Research studies had indicated that these compounds are endowed with several pharmacological properties[27,28]. Results of the investigation on the tooth ache activity of guava leaves carried out by Jayakumari[29] support the notion that plant flavonoids may have many roles as pharmaceuticals. Some of the biological functions affected by the flavonoids include capillary permeability, inhibition of enzymes, receptors and carriers[30,31]. Many research studies have demonstrated the antimicrobial activities of flavonoids against bacteria and yeast[32-34].

The commercial antibiotics and the guava leaf extracts investigated in our study have demonstrable antibacterial activities against diarrhea causing agents. However, the reports in the recent years on the rapid development of antibiotic resistance among different bacteria and the complications caused by their continual usage cannot be avoided. Moreover, action of these antibiotics has been observed to be unstable and uneven towards all the tested organisms. Thanangkol and Chaichangptipayut[35] had established the reliability and higher efficiency of guava leaves over commercial antibiotics in the treatment of acute diarrhea in humans.

CONCLUSION

The antibiotics currently used for treating infectious diarrhea although found effective, development of resistance towards these drugs by bacteria is not uncommon. The methanol extract of *P. guajava* L. as demonstrated in our study could be considered as a suitable and safe alternative to these drugs. It can be explored to develop a potential drug thus to combat these precarious bacterial pathogens implicated with diarrheal diseases afflicting millions of people in developing countries.

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