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Screening for antidiarrheal activity of *Psidium Guajava* : A possible alternative in the treatment against diarrhea causing enteric pathogens

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ABSTRACT
Psidium guajava leaves, commonly known as guava was screened against some common enteric pathogens like Escherichia coli, Shigella spp, Salmonella spp, Aeromonas spp, Staphylococcus aureus and Candida spp. The alcoholic and aqueous extracts of Psidium guajava leaves were obtained using the Soxhlet apparatus. Antimicrobial activity of the extracts were tested on Mueller Hinton Agar by the punch well technique. Significant inhibitory effects were observed against the isolates that were tested. Significant activity of ethanolic extract was observed against Vibrio, Aeromonas, Salmonella and Shigella species. Aqueous extract showed significant activity against candida and Escherichia coli. The results of the present study supported the claims of folk medicine for the use of this plant for the treatment of diarrhea. These results were encouraging and can be extrapolated further to consider the use of Psidium guajava leaf extracts as an alternative treatment option for diarrhea caused by the enteric pathogens.

Keywords: *Psidium guajava*, leaf extracts, Antimicrobial activity.

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INTRODUCTION

Since ancient times, diarrhea has been recognized as one of the critical health problem affecting mankind. Morbidity and mortality due to diarrhea continues to be a major problem particularly in those populations of socio-economically backward and developing countries. Infections like gastroenteritis and diarrhea are primarily associated with enteric bacteria most common of which are *Escherichia coli*, *Vibrio cholerae, Aeromonas spp., Shigella spp., Salmonella spp., Staphylococcus aureus, Campylobacter spp., Klebsiella spp.,* and *Pseudomonas spp.*

In developing countries like India, the low income group such as farmers, people from the isolated rural areas and native communities use folk medicine for the treatment of common infections [1]. When people from these remote communities get an infectious disease, they are usually treated by traditional healers and shamans because of their expertise in such procedures as making diagnoses, and treating them by the use of herbal medicines [2]. The use of medicinal plants as traditional medicines is well known in rural areas of many developing countries [3,4]. Synthesis of medicinally important compounds is very difficult and thus the cost of medicine is also high because of the non-availability of source materials especially aromatic compounds. The plants are the potential source of many drugs; but they did not yet get proper recognition. Many natural phenolic compounds found in plants and fruits are known to have antibiotic and antioxidant properties [5]. Traditional healers claim that their medicine is cheaper and more effective than modern medicine [6].

Antibiotic resistance is a major clinical problem in treating infections caused by these microorganisms. One way to prevent antibiotic resistance of the pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents [7]. Traditional healers claim that some medicinal plants are more efficient in treating infectious diseases than synthetic antibiotics. It is necessary to evaluate, with a scientific basis, the potential use of folk medicine for the treatment of diarrhea caused by the common enteric pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of diarrhea. They can also be a possible source for new potent antibiotics to which pathogenic strains may not be resistant[8]. Thus there is an immense necessity to seek the importance of natural plant remedies. Natural product may act as an alternative for antibiotics and chemotherapeutic agents in certain circumstances [9]. Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and wellbeing [10,11].

P. guajava has a rich ethno medicinal history. *P. guajava* is a member of the Myrtaceae family, which contains atleast 133 genera and more than 3,800 species. *P. guajava* is a large evergreen shrub or small tree that grows up to 15 metres in height. It is native to and widely distributed in Mexico and Central America. Today, the plant is cultivated from the west coast of Africa to the Pacific region, including India and China, with varieties originally introduced over the past 300 years from the United States [12] . Guava herbal medicine has been around and been used for ages. So it is important to analyze the antibacterial activity of *P.guajava* to find application in treatment for gastrointestinal ailments.

EXPERIMENTAL SECTION

Collection Of Plants:

The fresh and tender leaves of the plant *P. guajava* were collected from in and around Manipal, Udupi District, Karnataka, India. The taxonomic identities of the plant were confirmed by Dr. Richard Lobo, Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal. The vernacular name and their family names are mentioned below in Table 1.

Table 1. Ethno botanical information of *P. guajava*

Plant name	Common name	Family	Parts used
Psidium guajava	Guava	Myrtaceae	Tender leaves

The collected leaves were washed thoroughly under tap water followed by sterile distilled water for the removal of dust and soil particles. The leaves were shade dried for few days and then powdered.

Strains Used In The Study:

Bacterial strains used in the study were the isolates obtained from clinical samples at Kasturba hospital, Manipal and standard strains from National Institute of Cholera and Enteric Diseases (NICED) Kolkata, India. The lists of the organisms are given in Table 2.

Table 2.: List of microorganisms used in this study

S.NO	NAME
1	*Vibrio cholerae 01(el tor)*
2	*Vibrio parahemolyticus*
3	*Shigella species*
4	*Salmonella species*
5	*Enteropathogenic E. coli*
6	*Enterotoxigenic E. coli*
7	*Enteroaggregative E. coli*
8	*Aeromonas hydrophila*
9	*Candida species*

Preparation Of Extract:

Tender leaves of guava were shade dried and then coarse powdered. The powdered product was then subjected to exhaustive extraction with alcohol using Soxhlet apparatus [13], the extract obtained was then concentrated to remove the solvent residues and stored in a desiccator until use. The Aqueous extract of the leaves were prepared by cold maceration process [14] using chloroform-water mixture in the ratio 1:99 for 5-7 days. The extract obtained was filtered, concentrated and stored in a desicator until use.

Preparation Of Inoculum:

Stock cultures were maintained at 4°C on slopes of nutrient agar [15]. Active cultures for experiments were prepared by transferring a loopful from the stock cultures to test tubes of peptone water for bacterial isolates and Sabaurouds broth for *Candida*. The tubes were incubated without agitation for 24 hrs at 37°C. The cultures were diluted with fresh peptone water to achieve optical densities to 2.0×10^6 colony forming units (CFU/ml) [16]

Antimicrobial Activity:
The Agar diffusion (punch well) method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA) (dehydrated medium) obtained from Himedia (Mumbai). The test inoculums were swabbed uniformly onto the MHA plates and wells of diameter 6mm were punched out in each plate. 50 µl concentrations of alcoholic and aqueous extracts (1%, 3%, 5%, 7% and 9%) were loaded into the wells. The loaded wells were allowed to diffuse for 5 minutes and the plates were placed upright for incubation at 37°C for 24 hrs. At the end of incubation, inhibition zones formed around the wells were measured with transparent ruler in millimetres. Organism showing a clear zone of inhibition > 8 mm were considered to be sensitive. These studies were performed in triplicate and mean values were presented.

Sensitivity Of Bacteria To Standard Antibiotics:
The sensitivity pattern of the reference strains of bacteria were compared with the five commonly employed antibiotics, viz. ampicillin, cefixime, chloramphenicol, co-trimoxazole, gentamicin

RESULTS AND DISCUSSION

The multidrug resistance of microorganisms is a major medical concern, screening of natural products in a search for new antimicrobial agents that would be active against these microorganisms is the need of the hour [17]. So the study of antibacterial activity of guava leaves extract has been done and it showed significant results against many enteric pathogens tested. Table 3 summarizes the microbial growth inhibition of alcohol and aqueous extract of the screened plant species. It was observed that by gradually increasing the concentration of the extract the inhibitory zones also increased in atleast 9 out of the total 12 test strains.

In the present work the ethanolic extract of *P. guajava* showed better activity against the majority of the organisms tested in comparison with the aqueous extract. All the extracts showed varying degrees of antimicrobial activity on the microorganisms tested resulting in zone of inhibition of 10 – 31 mm. Aqueous extract of guava leaf showed maximum activity against *Candida albicans* and that of ethanolic was seen against *Aeromonas hydrophila* used in the study. *Vibrio cholera* and *Vibrio parahemolyticus* showed similar results. Moderate activity was observed with E.coli, salmonella and shigella strains. Most significant zones were observed with 7 and 9% of both Ethanolic and Aqueous extract.

Table 3 Inhibition zones produced by aqueous and ethanol extracts of guava leaves against few medically important enteric pathogens

Test Strains	Extracts acts	Concentration Of Extracts (%) Concentration Of Extracts (%)				
		1%	*3%*	*5%*	*7%*	*9%*
Vibrio cholerae	Aq	16	18	20	21	23
	Et	15	20	21	27	27
Vibrio parahemolyticus	Aq	15	16	19	21	21
	Et	16	17	19	24	26
Aeromonas hydrophila	Aq	15	17	19	21	22
	Et	26	27	29	29	31
ETEC	Aq	12	12	13	14	14
	Et	14	15	18	20	21
EPEC	Aq	15	17	18	19	20
	Et	11	12	12	14	14
EAEC	Aq	14	16	18	19	20
	Et	11	12	12	14	15
Salmonella typhimurium	Aq	12	13	13	15	15
	Et	12	12	13	15	16
Shigella flexneri	Aq	12	14	14	15	15
	Et	12	12	14	16	17
Shigella dysentriae	Aq	12	14	15	20	21
	Et	14	16	19	21	21
Shigella sonnei	Aq	15	16	18	19	19
	Et	15	15	17	19	20
Shigella boydii	Aq	14	15	15	19	20
	Et	17	20	21	23	24
Candida albicans	Aq	23	26	27	28	30
	Et	10	16	19	19	20

CONCLUSION

Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases [18]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that the plant extracts or their active constituents are used as folk medicine in traditional therapies by 80% of the world's population. There are about 45,000 plant species in India with the capacity to produce a large number or organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity [19,20]. The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects in comparison with the synthetic antimicrobials [21].

In conclusion these plants could be a potent source of new antibiotic compounds. Further work is needed to isolate the secondary metabolites from the extracts studied in order to test specific antimicrobial activity. Further research is also necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of in vitro antimicrobial evaluation of the tender leaves of guava plants form a primary platform for further phytochemical and pharmacological studies. In the present study, we have found that the biologically active phytochemicals were present in the methanol extracts . Further studies are required and are in progress in many laboratories to isolate the active components.

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