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Research Article

Studies On Effect of Guava Leaves Extract Against Selected Enteric Bacteria

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ABSTRACT

In the present work we evaluated the antibacterial activity of crude Guava (*Psidium guajava*) leaves extract in comparison with Standard antibiotics Cholistin, Erythromycein, Ciprofloxin, Methicillin, Ampicillin and Cephalosporin. For the present study following bacterial cultures were used those were *Salmonella typhi* (8 strains), *Shigella dysenteriae* (7 strains), *Salmonella paratyphi A* (5 strains), *Salmonella paratyphi B* (7strains), *E.coli* (4 strains), *Citrobactor spp.* (3 strains).

The antimicrobial activity of Crude Guava leaves extract was represented in table 1. Guava leaves extract showed highest antibacterial activity against *Salmonella typhi* strain I (ZOI-20mm), *Shigella dysenteriae* strain I (ZOI- 15mm), *Salmonella paratyphi A* strain II (ZOI-18mm), *Salmonella paratyphi B* strain V (ZOI- 20mm), *E.coli* strain III (ZOI- 14mm) and *Citrobactor spp.* strain III (ZOI- 20mm).

Salmonella typhi strain V was observed sensitive to antibiotic Ciprofloxin (ZOI- 12mm). *Shigella dysenteriae* strain IV was observed sensitive to antibiotic Ampicillin (ZOI- 13mm) and Erythromycein (ZOI- 16mm). *Salmonella paratyphi A* strain III was observed sensitive to antibiotic Ciprofloxin (ZOI- 15mm) and Ampicillin (ZOI- 14mm). *Salmonella paratyphi B* strain V was observed sensitive to antibiotic Ciprofloxin (ZOI- 18mm) and Ampicillin (ZOI- 15mm).

E.coli strain I was observed sensitive to antibiotic Cholistin (ZOI- 14mm), Ampicillin (ZOI-10mm), Cephalosporin (ZOI- 17mm) and Erythromycein (ZOI- 15mm). *Citrobactor spp.* strain I, was observed sensitive to antibiotic Cholistin (ZOI- 11mm). The results of this study showed that some pathogenic enteric bacteria were observed resistant to standard antibiotics but they were observed sensitive to the crude Guava leaves extract. The results of the present study also support the medicinal usage of the Guava leaves.

Key-words: Guava (*Psidium guajava*), Antimicrobial activity, Enteric pathogens, Standard antibiotics etc.

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INTRODUCTION

India is one of the pioneers in the invention of herbal medicines for the treatment of diverse ailments. In the ancient past, India exported several such drug plants to the oriental countries as well as to Greece, Italy, Egypt and Arab countries. With the improvement of Western therapy people lost importance in herbal medicines. In recent years this tendency has reversed. Severe research in this area is now being pursued all over the world. Serious efforts are being made by the phytochemists and botanists in exploring the plant world to find out more effective drugs from botanical species. These studies have culminated in the isolation, structure elucidation and synthesis of several biologically active compounds.

Medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic importance. These plants are economical and renewable sources of pharmacologically-active substances and are recognized to produce certain chemicals that are naturally toxic to bacteria. The increase in antibiotic resistant bacteria is largely due to the extensive use of antibiotics in medicine, in animal care and in agriculture. The difficulty is compounded by the lack of new antibiotics to attack bacteria in different ways to avoid the resistant genes. Decreasing effectiveness and resistance of pathogens to antimicrobial drugs made the search of a new antimicrobial agent an important strategy for the organization of substitute therapies in difficult handling infections.

In the last few years, a number of studies have been conducted in different countries to verify such efficiency. Antibiotics supply the main source for the therapy of microbial (bacterial and fungal) infections. Since the detection of these antibiotics and their uses as chemotherapeutic agents there was a confidence in the medical society that this would lead to the ultimate eradication of infectious diseases. Herbal medicines previously form the basis of therapeutics used in developing countries but recent years have also seen an increase in the use of herbal medications in the developed world as well. Some studies focusing on the examination of traditional African [1], Caribbean [2] and Indian [3] medicinal plants have resulted in the recognition of new sources of therapeutic agents.

Antimicrobial multiple drug resistance towards commonly used commercial drugs has resulted in an increase in the look for antimicrobial agents from natural sources. Plant resulting antimicrobial agents are a largely ideal resource with enormous medical prospective and much more study is needed in this area. Infective diseases account for approximately one-half of all deaths in tropics [4]. In the area of anti-infective about 70% are naturally derived [5]. The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic chemotypes. Herbal and natural products of folk medicines have been used for centuries in every culture all over the world. Scientists and medical professionals have shown increased interest in this field as they recognize the true health reimbursement of these remedies.

Plants are a wealthy source of secondary metabolites with attractive biological actions. In general, these secondary metabolites are a key source with a range of structural arrangements and properties [6]. Natural products from microbial sources have been the principal resource of antibiotics, but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very important because these may provide as talented sources of book antibiotic prototypes [7]. It has been shown that *in vitro* screening methods could provide the required preliminary observations essential to select crude plant extracts with potentially useful properties for additional chemical and pharmacological investigations [8].

Guava leaves have long been recognized for their antimicrobial activity. *Psidium guajava* L, (*Myrataceae*) is one of the parts in folk medicine that has been used for the management of various disease conditions and is believed to be active. Various parts of the plant has been used in traditional medicine to manage conditions like malaria, gastroenteritis, coughs, sore throat, inflamed gum and other conditions [9-11]. Thus it is used in traditional medicine is well established against enteric human bacteria. The morphology of the plant has been described severally in literature [12-14].

MATERIALS AND METHODS

Collection of Plant Material

Healthy disease free, indigenously grown mature leaves of Guava was collected from local area of Solapur (M.S.). The identification of plant material was confirmed by a Botanist in the Dept. of Botany, Walchand College of Arts and Science, Solapur (M.S.).

Test Pathogens

Various strains of Enteric pathogenic bacterial cultures were used in this study. Those were *Salmonella typhi* (8 strains), *Shigella dysenteriae* (7 strains), *Salmonella paratyphi A* (5 strains), *Salmonella paratyphi B* (7strains), *E.coli* (4 strains), *Citrobactor spp.* (3 strains). The pure pathogenic bacterial strains were collected from Dept. of Microbiology, V.M. Govt. Medical College, Solapur (M.S.) and Ashwini Sahakari Rugnalaya NYT. And Research Centre, Solapur (M.S.). The cultures were isolated and identified by using standard biochemical tests. The cultures were maintained on nutrient agar slants at 4^0C and subcultured for 24hr. before use.

Preparation of Leaves Extracts

Thoroughly washed mature leaves of Guava plants were shade dried and then powdered with the help of electric blender. Twenty five gram powder was put into boiling water and allowed to settle for 2hr. After settling the extract was collected and preserved at 5^0C in airtight bottle until further use.

Antibacterial Activity Assay

Antimicrobial activity of the Guava leaves extract was determined by agar well diffusion method on Muller- Hinton agar medium [15]. Cups are made on Muller- Hinton agar plates using cork borer and inoculum containing 10^6 CFU/ml of pathogenic bacteria were spread on the solid plate with the help of sterile glass rod. Then 100ul of crude Guava leaves extract was placed in the cups made in inoculated plates. All the plates were incubated for 24hr. at 37^0C.and after incubation period zone of inhibition was measured in mm. Antimicrobial activity of Standard antibiotics Cholistin, Erythromycein, Ciprofloxin, Methicillin, Ampicillin and Cephalosporin were also observed in comparison with Guava leaves extract.

STATISTICAL ANALYSIS

The resultant clear zones around the well were measured in mm. The antibacterial activity of crude Guava leaves extract was indicated by clear zones of growth inhibition. Three replicates were maintained for each treatment. Each value represents mean of three different observations ± S.D. The data were subjected to statistical analysis as per the method of Gomez and Gomez [16].

RESULTS

In the present study significant antibacterial activity is observed by crude Guava leaves extract. The antimicrobial activities of Guava leaves extract was represented in table 1. Guava leaves extract showed highest antibacterial activity against *Salmonella typhi* strain I (ZOI- 20mm), *Shigella dysenteriae* strain I (ZOI- 15mm), *Salmonella paratyphi A* strain II (ZOI- 18mm), *Salmonella paratyphi B* strain V (ZOI- 20mm), *E.coli* strain III (ZOI- 14mm) and *Citrobactor spp.* strain III (ZOI- 20mm). *Salmonella typhi* strain V was observed sensitive to antibiotic Ciprofloxin (ZOI- 12mm), strain VIII was observed sensitive to antibiotic Ciprofloxin (ZOI-14mm), strain II was observed sensitive to antibiotic Ciprofloxin (ZOI- 16mm), and strain IV was observed sensitive to antibiotic Ciprofloxin (ZOI-12mm). Rest of all strains was observed resistant to all tested antibiotics.

Shigella dysenteriae strain IV was observed sensitive to antibiotic Ampicillin (ZOI- 13mm) and Erythromycein (ZOI-16mm). Rest of all strains was observed resistant to all tested antibiotics. *Salmonella paratyphi A* strain III was observed sensitive to antibiotic Ciprofloxin (ZOI- 15mm) and Ampicillin (ZOI- 14mm). Rest of all strains was observed resistant to all tested antibiotics. *Salmonella paratyphi B* strain V was observed sensitive to antibiotic Ciprofloxin (ZOI- 18mm) and Ampicillin (ZOI- 15mm). Rest of all strains was observed resistant to all tested antibiotics.

E.coli strain I was observed sensitive to antibiotic Cholistin (ZOI- 14mm), Ampicillin (ZOI-10mm), Cephalosporin (ZOI- 17mm) and Erythromycein (ZOI- 15mm). *E.coli* strain II was observed sensitive to antibiotic Cholistin (ZOI-12mm), Ampicillin (ZOI- 12mm), Cephalosporin (ZOI- 18mm) and Erythromycein (ZOI- 14mm). *E.coli* strain III was observed sensitive to antibiotic Cholistin (ZOI- 16mm), Ampicillin (ZOI- 14mm), Cephalosporin (ZOI- 15mm) and Erythromycein (ZOI- 11mm), while *E.coli* strain IV was observed sensitive to antibiotic Cholistin (ZOI- 12mm), Ampicillin (ZOI- 15mm), Cephalosporin (ZOI- 16mm) and Erythromycein (ZOI- 13mm).

Citrobactor spp. strain I, II and III was observed sensitive to antibiotic Cholistin (ZOI- 11mm, 15mm and 16mm respectively), Erythromycein (ZOI-13mm, 12mm, 15mm respectively), Ampicillin (ZOI- 14mm, 15mm, 16mm

respectively), Cephalosporin (ZOI- 12mm, 15mm, 13mm respectively) and Methicillin (ZOI- 13mm, 14mm, 15mm respectively).

DISCUSSION

Medicinal plants are the boon of nature to cure a number of ailments of human beings. In many parts of the world medicinal plants are used against bacterial, viral and fungal infections. Evaluation of plants bearing efficiency in healing various diseases is growing in recent years. Innumerable biologically active compounds of plants are found to possess antibacterial properties. Practitioners of Ayurveda and Unani system of medicine regularly employ a large number of Indian medicinal plants as antibiotic agents and over the last 40 years, intensive efforts have been made to discover clinically used herbal antibacterial and antifungal drugs. The results obtained in this study suggest that the crude Guava leaves extract can be used as potential source of drugs in the treatment or control of intestinal disorders.

Table 1: Antimicrobial Activity of Crude Guava Leaves Extract

M/O	Strains with ZOI in mm (Mean ± SD)							
	I	II	III	IV	V	VI	VII	VIII
Sal.typhi	20±0.62	18±0.72	15±0.88	14±0.34	10±0.26	11±0.17	09±0.62	08±0.20
Shigella dysenteriae	15±0.62	14±0.34	13±0.17	10±0.26	14±0.34	12±0.17	14±0.43	-
Sal.para.A	16±0.17	18±0.26	10±0.52	12±0.17	14±0.20	-	-	-
Sal.para.B	10±0.26	14±0.55	16±0.62	18±0.72	20±0.62	14±0.10	16±0.55	-
E.coli	10±0.17	12±0.62	14±0.43	13±0.70	-	-	-	-
Citrobactor spp.	15±0.62	18±0.26	20±0.20	-	-	-	-	-

(- = No Strians)

Fig 1: Graphical Representation of Antimicrobial Activity of Crude Guava Leaves Extract.

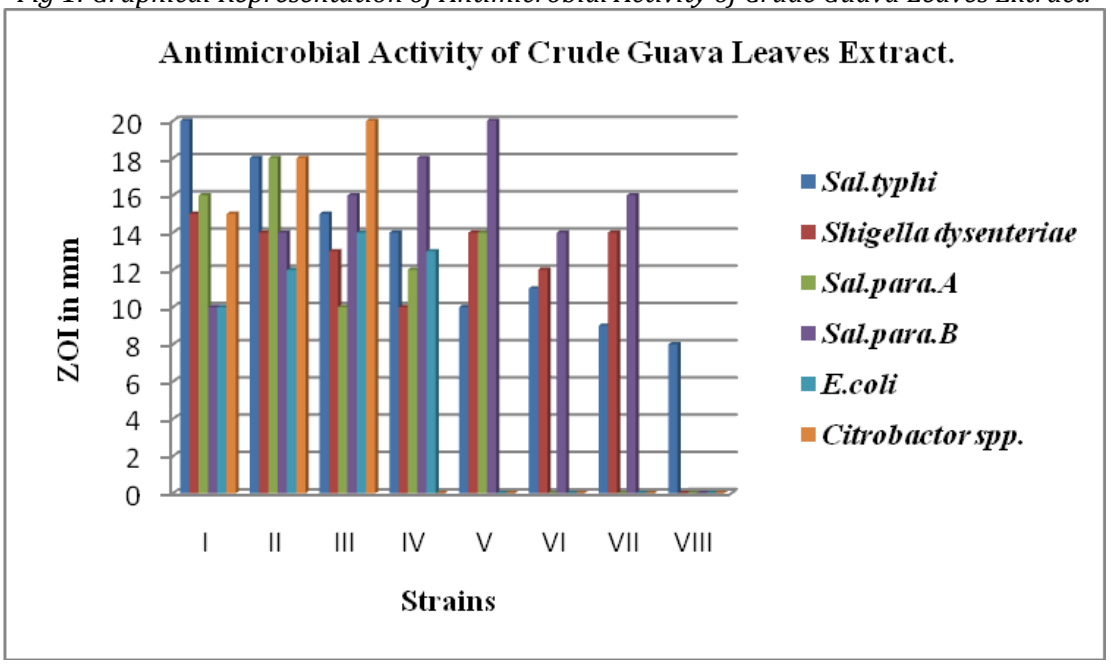

Table 2: Antimicrobial Activity of Standard Antibiotics against Enteric Pathogens

M/O	Strains with ZOI in mm							
	I	II	III	IV	V	VI	VII	VIII
Sal.typhi	®	16(Cipro)	®	12 (Cipro)	12 (Cipro)	®	®	14(Cipro)
Shigella dysenteriae	®	®	®	13(Amp) 16(Ery)	®	®	®	-
Sal.para.A	®	®	15(Cipro) 14(Amp)	®	®	-	-	-
Sal.para.B	®	®	®	®	18(Cipro) 15(Amp)	®	®	-
E.coli	14(Cho)10(Amp)17(Ceph15(Ery)	12(Cho) 12(Amp)18(Ceph)14(Ery)	16(Cho) 14(Amp) 15(Ceph) 11(Ery)	12(Cho) 15(Amp) 16(Ceph) 13(Ery)	-	-	-	-
Citrobactor spp.	11(Cho)13(Ery) 14(Amp) 12(Ceph) 13(Met)	15(Cho) 12(Ery) 15(Amp)15(Ceph)14(Met)	16(Cho) 15(Ery) 16(Amp) 13 (Ceph) 15(Met)	-	-	-	-	-

(Cho- Cholistin, Ery- Erythromycein, Cipro- Ciprofloxin, Met- Methicillin, Amp- Ampicillin Ceph- Cephalosporin, ®- Resistant Strain, - = No Strians).

CONCLUSION

Earlier phytochemical review reported that leaves of *Psidium guajava* (Guava) contains high amount of flavonoids. Leaves of *Psidium guajava* is a rich source of quercetin. Quercetin is effective in free radical scavenging property, and has analgesic, anti-inflammatory, antibacterial properties. *P. guajava* tree has a long history of medicinal uses that are still employed today [17]. The presence of pharmacologically useful substances such as alkaloids, tannins, flavonoids, saponins among others in the leaves confirm the diverse claims and application of parts of the plant in local treatment of ailments. The present study suggested that, the crude Guava leaves extract has a great potential as antimicrobial agent against selected MDR enteric pathogens in comparison with selected standard antibiotics and can be used as an alternative medicine in the treatment of enteric disorders. The antimicrobial activity assays showed promising evidence for the antimicrobial activity of Guava leaves extract against selected enteric pathogens. Therefore, the Guava leaves extract could be seen as a good source for useful drugs.

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