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Research Article

Screening of Guava (*Psidium gaujava)* for Effective Phytomedicines and Study on its Antimicrobial effect against Selected Enteric Pathogens

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ABSTRACT

The present study was carried out for determination of antibacterial activity and phytochemical screening of Guava *(Psidium guajava)* leaves solvent extracts. Four solvents were used for extraction. Those were Ethanol, Methanol, Diethyl ether and Acetone. The solvent extraction was done by using Soxlet apparatus. Test microorganisms were screened to confirm their viability and identities using standard microbiological methods. The different solvent extracts of Guava leaves was tested for antimicrobial activity using the standard agar well diffusion method against nine enteric pathogens, these are *E.coli, Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B, Shigella sonnei, Shigella dysentarie, Enterobactor spp.,Citrobactor spp.and Klebsiella spp.* The Ethanol extract of Guava leaves showed highest antimicrobial activity against *Salmonella paratyphi A.* The Methanol extract of Guava leaves showed highest antimicrobial activity against *Citrobactor spp.* The Diethyl ether extract of Guava leaves showed highest antimicrobial activity against *Klebsiella spp.* while Acetone extract showed highest antimicrobial activity against *Shigella dysentarie.* The antimicrobial activity of standard antibiotics Ampicillin and Tetracycline was studied in comparison with Guava leaves solvent extracts.

The MIC values were determined by both agar and broth dilution method. The functional chemical group was determined by Fourier Transform Infrared Spectroscopy (FTIR).The phytochemical analysis of Guava leaves solvent extracts showed presence of Alkaloid, Flavonoid, Tanin, Phenolic compounds, Terpenoid etc.

Keywords: Guava *(Psidium guajava),* Antimicrobial activity, Phytochemical screening, Enteric pathogens, MIC, FTIR etc.

INTRODUCTION

Since ancient times, plants have been a veritable source of drugs. Research work on medicinal plants are intensified and information of these plants be exchanged. This thought will go a long way in scientific exploration of medicinal plants for the benefit of man and is likely to decrease the dependence or importance of drugs. Because of the pathogenic microorganisms are developing the resistance to current antibiotics, there is a need for the search of new antimicrobial agents mainly among plant extracts.

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. In traditional medicine, various plant parts has been used to control malaria, gastroenteritis, coughs, sore throat, inflamed gum [1-3]. 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 [4-6].

This extensive flora has been greatly utilized as a source of many drugs in the Indian traditional system of medicine[7]. About 80% of the world population depends on herbal based alternative systems of medicine. The activities of these curative plants are evaluated by their chemical components. Indian Ayurveda utilizes about 2000 plants to cure different ailments[8]. The ethnobotanical studies and folklore claiming reviewed that the leaves of the Guava was used for antioxidant, hepatoprotective, anti- allergy,

antimicrobial, antigenotoxic, anti plasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, anti- inflammatory and anti nociceptive activities. Guava leaf tea is commonly used as a medicine against gastroenteritis (dysentry) and child diarrhea [9].

WHO (world health organization) says that plants would be the best source for obtaining different types of medicines and drugs. These natural products are widely used by human with its effective results. Extract from Guava leaves mostly contain essential oil, tannins, flavonoids, phenol compounds, carotenoids and vitamin C. Guava leaf extracts introduces many biological activities i.e. Antibacterial, antioxidant, and analgesic, anti inflammatory, antimicrobial, phytotoxic, hepatoprotection, and anti hyperglycaemic and anti cancer activities [10].

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:

Nine strains of enteric pathogenic bacterial cultures were used in this study. Those were *E.coli*, *Salmonella typhi*, *Salmonella paratyphi A*, *Salmonella paratyphi B*, *Shigella dysenteriae*, *Shigella sonnei*, *Enterobactor spp.*, *Citrobactor spp.* and *Klebsiella spp.* The pure pathogenic bacterial strains were collected from Dept. of Microbiology, V.M.Govt.Medical College,Solapur (M.S.).The collected pure pathogenic bacterial strains were isolated from hospitalized patients at Govt.Civil Hospital, Solapur (M.S.) The cultures were maintained on nutrient agar slants at 4^0C and subcultured for 24hr. before use.

Preparation of Solvent Extracts:

Thoroughly washed mature leaves were shade dried and then powdered with the help of electric blender. Twenty five grams of the powder was filled in the thimble and extracted successively with Ethanol, Methanol, Diethyl ether and Acetone using a Soxlet extractor for 48hr.All the extracts were concentrated using rotary flash evaporator and preserved at 5^0C in airtight bottle until further use. All the extracts were subjected to antibacterial activity assay and phytochemical analysis.

Antibacterial Activity Assay:

Antimicrobial activity of the Guava leaves solvent extracts was determined by agar well diffusion method on Muller- Hinton agar medium [11] .Cups was made on Muller- Hinton agar plates using cork borer and inoculum containing 10^6 CFU/ml of pathogenic bacteria was spread on the solid plate with the help of sterile glass rod. Then 100ul of solvent 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 Ampicillin and Tetracycline were observed in comparison with Guava leaves solvent extracts.

Determination of Minimum Inhibitory Concentration (MIC):

MIC was determined by both agar and broth dilution methods [12]. For broth dilution tests, 0.1ml of standardized suspension of bacteria (10^6 CFU/ml) was added to each tube containing different concentrations of solvent extracts (05-50ul/ml) and incubated for 24hr at 37^0C.In agar plating method dilutions having 05-50ul of solvent extracts was placed in the cups on the inoculated plates and incubated as mentioned above.

Phytochemical Analysis:

Qualitative Phytochemical Analysis:

The Guava leaves solvent extracts was tested for the presence of bioactive compounds by using standard method [13].

Fourier Transform Infrared Spectroscopy (FTIR):

FTIR was used to identify the characteristic functional group in the crude Guava leaves powder. A small quantity (5mg) of the powder was dispersed in dry potassium bromide (Kbr). The mixture was thoroughly mixed in a mortar and pressed at pressure of 6 bars within 2 min. to form a Kbr thin disc. Then the disc was placed in a sample cup of a diffuse reflectance accessory. The IR spectrum was obtained using Perkin Elmer 2000 infrared spectrometer. The sample was scanned from 4000 to 400cm^{-1} for 16times to increase the signal to noise ratio.

RESULTS AND DISCUSSION

In the present study significant antibacterial activity is observed by all solvent extracts of Guava leaves. The antimicrobial activity of Guava leaves solvent extracts were represented in table 1.The results revealed that the Ethanol extract of Guava leaves shows highest antibacterial activity against *Salmonella para.A.* The Methanol extract of Guava leaves shows highest antibacterial activity against *Citrobactor spp.*

The Diethyl ether extract of Guava leaves shows highest antibacterial activity against *Klebsiella spp.* while Acetone extract of Guava leaves shows highest antibacterial activity against *Shigella dysenteriae*. The antimicrobial activity of standard antibiotic Ampicillin was found to be maximum against *Citrobactor spp.* while standard antibiotic Tetracycline showed highest antibacterial activity against *Salmonella para.A.*

Minimum inhibitory concentration (MIC) of the different Guava leaves solvent extracts varied against different test pathogens. The MIC of Guava leaves solvent extracts required for test pathogens were represented in table 2. Lowest MIC of 5ul was observed against *Shigella sonnei* by Ethanol extract of Guava leaves while highest MIC of 40ul was observed against *E.coli.* Lowest MIC of 10ul was observed against *Citrobactor spp* by Methanol extract of Guava leaves while highest MIC of 50ul was observed against *Salmonella typhi.* Lowest MIC of 5ul was observed against *Klebsiella spp* by Diethyl ether extract of Guava leaves while highest MIC of 50ul was observed against *Citrobactor spp.* Lowest MIC of 5ul was observed against *Shigella dysenteriae* by Acetone extract of Guava leaves while highest MIC of 50ul was observed against *Salmonella typhi.*

The aim of FTIR analysis is to determine the existence of functional group that exists on isolate. The IR spectrum of the crude powder of Guava leaves in the form of Kbr pallet is shown in fig 1.

The absorption at 3425cm^{-1} is due to presence of hydroxyl group. The absorption at 1634cm^{-1} is due to c=c stretching .The absorption at 1321 cm^{-1} is due to bending vibrations of C-H bonds of methyl group.

The results of phytochemical analysis of various solvent extracts of Guava leaves were represented in table 3. The phytochemical analysis showed presence of Alkaloid, Flavonoid, Tanin , Phenolic compounds, Terpenoid etc.

Successful prediction of antimicrobial compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Traditionally, medicinal plants was primarily extracted in water as the solvent but in our studies we found that plant extracts in organic solvents provided more reliable antimicrobial activity compared to previous studies those extracted in water. These observations can be rationalized in terms of the polarity of the compounds being extracted by each solvent and in addition to their intrinsic bioactivity. Ethanol, Methanol, Diethyl ether and Acetone extract of Guava leaves showed pronounced activity against all the tested microorganisms. Guava leaf extract have been shown to be effective against many bacterial species known to cause diarrhea, including *S. aureus, E. coli* and other common enteropathogenic cultures. Guava plant contains tannins, phenols, triterpins, flavonoids, essential oils, saponins, carotenoids, lectins and all those compounds together acts as antimicrobial agents [10]. Past research results indicate the presence of polyphenolic compounds like quercetin, avicularin and guaijaverin being the active antimicrobial components. These results support the traditional information of local users about their collection of plant samples as antimicrobial agents. To support proper preservation and sustainable use of such plant resources, knowledge of local communities should be enhanced incorporating the habitual knowledge with scientific conclusion. The results of the present study also support the medicinal usage of the studied plants and propose that these extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the treatment of infectious diseases. The extract showing highest antimicrobial activity can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological assessment.

CONCLUSION

The results of present study evidenced that studied Guava leaf solvent extracts have the ability to inhibit the growth of the selected enteric pathogens with its abundant source of secondary metabolites. This also helps to become an alternate and minimize the excessive of antibiotics for the prevention of enteric disorders. The present study suggested that, the various solvent extracts of Guava leaves have a great prospective as antimicrobial agents against selected enteric pathogens and they can be used as an alternative medicine in the treatment of enteric disorders. The antimicrobial activity and MIC assays showed promising evidence for the antimicrobial activity of Guava leaves solvent extracts against selected enteric pathogens. Phytochemical analysis showed presence of antimicrobial substances in the studied extracts. The results revealed the presence of medicinally important constituents in these solvent extracts. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Therefore, the Guava leaves solvent extracts could be seen as a good source for useful drugs.

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Table1
Antibacterial Activity of Guava Leaves Solvent Extracts.

Microorganism	Zone of Inhibition (mm)					
	Ethanol	Methanol	D.E	Acetone	Ampi	Tetra
E.coli	3	5	3	4	13	14
Sal.typhi	3	3	4	3	14	11
Sal.para A	7	4	5	5	10	15
Sal.para B	6	4	3	3	11	12
Shigella sonnei	5	5	5	6	06	12
Shigella dysenteriae	4	4	4	7	12	11
Enterobactor spp.	6	3	5	5	12	14
Citrobactor spp.	4	6	3	3	15	13
Klebsiella spp.	5	3	7	6	09	11

(D.E.-Diethyl ether, Ampi- Ampicillin, Tetra- Tetracycline)

Table 2
MIC of Guava Leaves Solvent Extracts.

Microorganism	Minimum Inhibitory Concentration (ul/ml)			
	Ethanol	Methanol	D.E	Acetone
E.coli	40	25	45	30
Sal.typhi	20	50	15	50
Sal.para A	10	35	25	20
Sal.para B	10	25	40	45
Shigella sonnei	05	20	30	10
Shigella dysenteriae	30	20	35	05
Enterobactor spp.	25	45	10	45
Citrobactor spp.	35	10	50	40
Klebsiella spp.	25	40	05	15

(D.E. - Diethyl ether)

Table3
Phytochemical Analysis of Guava Leaves Solvent Extracts.

Test	Ethanol	Methanol	D.E	Acetone
Alkaloid	+	+	+	+
Flavonoids	+	+	-	+
Tanin	-	+	+	-
Saponins	-	+	-	-
Carbohydrate	+	+	+	+
Phenolic Compounds	+	+	+	+
Terpenoid	+	-	-	-

(+ = Present, - = Absent)

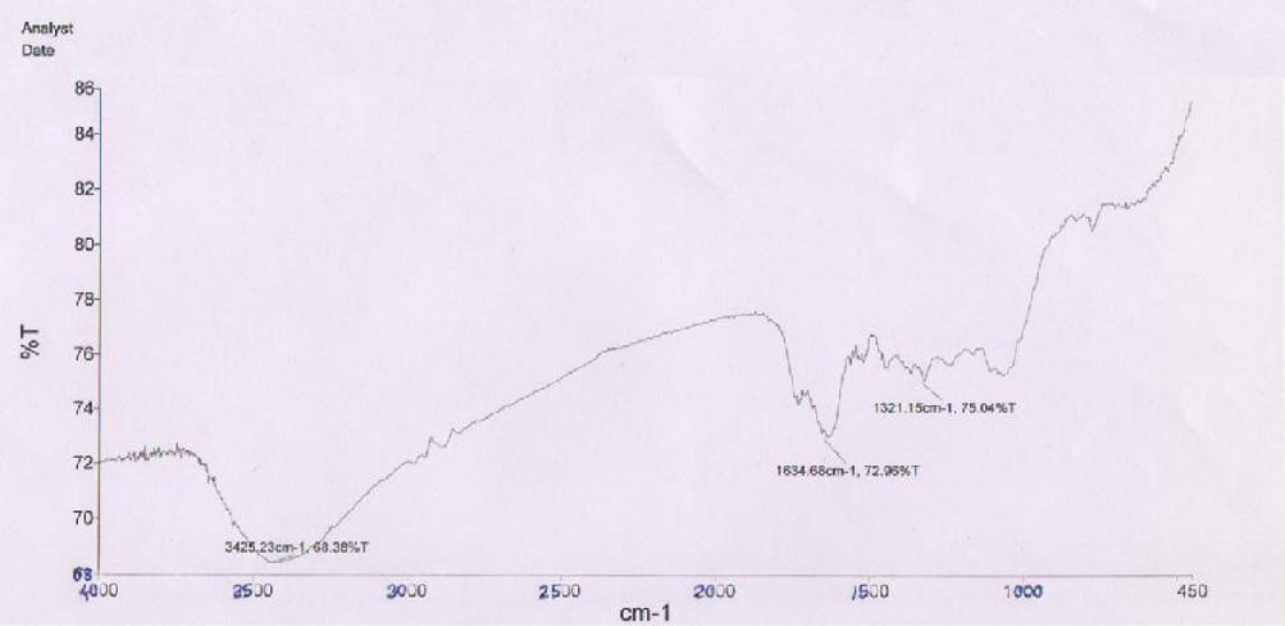

Fig1
Fourier Transform Infrared Spectroscopy (FTIR) Analysis of Crude Guava Leaves.

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