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ANTIMICROBIAL POTENTIAL OF LEAVES OF *PSIDIUM GUAJAVA*

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ABSTRACT
The aim of the study was focused on the antibacterial potential of dried leaves solvents extracts of five different extracts (acetone, chloroform, methanol, petroleum ether and water) of *Psidium guajava*(family Myrtaceae) were tested against *Bacillus cereus*, *Bacillus subtilis*,*Bacillus megaterium, Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris* and *Pseudomonas aeruginosa* by well diffusion method.Acetone, methanol and petroleum ether extracts showed significant antibacterial activity.All the organisms are found susceptible except *Bacillus cereus*. Chloroform extract is active against only *Bacillus subtilis*. The fresh leaves juice was found to be active against all organisms except *Pseudomonas aerugenosa* and the aqueous extract found to be active against *Proteus vulgaris* and *Staphylococcus aureus*. Results were compared with standard antibiotics Amoxicillin-Am^{30}, Ciprofloxacin-Cf^{30}, Cotrimaxazole-Co^{25},Gentamicin-G^{50} and Tetracycline-T^{30}.

INTRODUCTION

In developing countries,the frequency of life threatening infections were caused by pathogenic microorganisms has led to increased worldwide and in becoming an important cause of morbidity and mortality in immunocompromised patients (Al-Bari *et al.,* 2006). The historical point, plants have been used as an important source of natural products for human health. All over the world, the antimicrobial properties of plants have been investigated by a number of studies and many of them have been used as therapeutic alternatives because of their antimicrobial properties (Adriana *et al.,* 2007) and they contain secondary metabolites such as alkaloids,phenolic compounds,etc. The practice of complementary and alternative medicine is now on the increase in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that have provided the scientific basis for the efficacy of many plants used in folk medicine to treat infections (Vijaya and Ananthan, 1997; Dilhuydy, 2003). It is therefore very necessary that the search for newer antibiotic sources be a continuous process.Plants are the cheapest and safer alternative sources of antimicrobials (Pretorius and Watt, 2001; Doughari *et al.,* 2007). According to World Health Organization (Santos *et al.,* 1995) medicinal Plants would be the best source to obtain a variety of drugs.About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Ellof, 1998). In India thousands of species are known to have medicinal values and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient times (Parekh *et al.,* 2005) Medicinal plants are valuable natural resources and regarded as potentially safe drugs and have been tested for biological, antimicrobial and hypoglycemic activity also play an important role in modern medicine (Hassawi and Kharma, 2006; Bhat *et al.,* 2009). It is well known that even the most synthetic drugs have their origin from plant products (Sofowara, 1982). The screening of plant extracts and their products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotics prototypes (Afolayan, 2003) the selection of crude plant extracts for screening programs is potentially more successful in initial steps than the pure compounds (Kasamato *et al.,* 1995). Such screening of various plant extracts has been previously studied by many workers (Erdogrul, 2002; Parekh and Chanda, 2007). Eventhough hundreds of plant species have been tested for antimicrobial properties,the vast majority of them have not yet been evaluated.

Psidium guajava L, commonly known as guava, of the family Myrtaceae, is a native plant of India. Different parts of the plant are used in the indigenous system of medicine for the treatment of various human ailments such as wounds, ulcers, bowels and cholera(Begum *et al.,* 2002).Pharmacological investigations indicated that its bark, fruit, and leaves possess antibacterial, hypoglycemic, anti-inflammatory, analgesic, antipyretic, spasmolytic and CNS depressant activities (Begum *et al.,* 2002). In Mexico, *P. guajava* leaves are extensively used to stop diarrhea and the quercetin and its glycosides were its active compounds. The leaves of P.guajava contain an essential oil rich in cineol, tannins, triterpenes and flavonoids (Olajide *et al.,* 1999).

In vitro studies on plants used in traditional medicine have been carried out in the field of microbiology, especially on pathogenic bacterial growth; and some studies were about

the antimicrobial activity of *Psidium guajava* (Gnan and Demello, 1999; Jairaj *et al.,* 1999; Nascimento *et al.,* 2000; Ahmad and Beg, 2001; Abdelrahim *et al.,* 2002; Holetz *et al.,* 2002; Voravuthikunchai *et al.,* 2004; Qadan *et al.,* 2005).

The present study was undertaken to investigate the in vitro antibacterial activity of water, acetone, chloroform, methanol and petroleum ether extracts from leaves of *Psidium guajava*.

MATERIALS AND METHODS

Selection of medicinal plant for the study

Identification and preservation of plant materials

Fresh plant leaves of *Psidium guajava* were collected from the Nagpur area and identified.Plant leaves were washed with 70% alcohol and then rinsed with sterilized distilled water, air dried and stored in airtight bottles at 4ºC for further use.

Preparation of crude extract

Homogenized mass of leaves was squeezed in 400 mesh nylon cloth (pore size 37 micron) to obtain crude extract. Crude extract was prepared fresh and used before 2h. Cold extracts were prepared using individual fresh plant leaves.

Crude extraction

Aqueous extraction

Ten gram of dried powder was extracted in 100 mL distilled water for 6 h at slow heat. Every 2 h, it was filtered through 8 layers of muslin cloth and centrifuged at 5000g for 15 min. The supernatant was collected. This process was repeated twice and after 6 hr, the supernatant was concentrated to make the final volume one-fourth of the original volume (Shahidi Bonjar, 2004). It was then autoclaved at 121ºC and 15 lbs pressure and then stored at 4ºC.

Solvent extraction

Ten gram of dried powder was extracted with 100 mL of each solvent (acetone, chloroform, methanol and petroleum ether) and flasks were kept on a rotary shaker at 190-220 rpm for 24 h. Thereafter, it was filtered through 8 layers of muslin cloth and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Shahidi Bonjar, 2004). It was stored at 4ºC in airtight bottles for further studies.

Bacterial cultures

The microbial strains are identified strains and were obtained from the National Chemical Laboratory (NCL),Pune, India. The studied bacterial strains were *Bacillus cereus* NCIM2155, *Bacillus subtilis* NCIM2063, *Bacillus megaterium* NCIM2087, *Escherichia coli* NCIM2931, *Proteus vulgaris* NCIM2857 and *Pseudomonas aeruginosa* NCIM5029. *Staphylococcus aureus* MTCC96 this strain was procured from Institute of Microbial Technology (IMTECH), Chandigarh,India.They were sub-cultured on nutrient agar for every 15 days and maintained on nutrient agar slants at 4ºC.

Media

Hi -Sensitivity test broth (M 486) and Hi-sensitivity test agar (M 485) were procured from Hi-media Mumbai,India. The media were prepared according to the instructions given.

Screening for the antimicrobial potential of the plant leaves extracts

The antimicrobial activity of different solvent extracts was evaluated by agar well diffusion (Perez *et al.,* 1990; Parekh *et al.,* 2006) using Hi-sensitivity test agar (M 485).

Preparation of inoculum

A loopful of culture was inoculated from the stock slant culture in 5 mL of Hi-sensitivity test broth and broth was incubated at 35 ± 0.5ºC in incubator for 18-20h. After incubation a loopful of actively growing culture was inoculated into 10 mL of Hi-sensitivity broth. Broth was incubated at 35 ± 0.5ºC for 6-8h. This culture was used for the inoculation of Hi-sensitivity test agar plates.

Preparation of hi-sensitivity test agar medium

Hi-sensitivity test agar medium was prepared as per instructions of manufacturer. Required amount of agar medium was melted and 25 mL of molten medium was distributed in test tubes (25x150 mm). Medium was autoclaved at 15 lb. for 20 min. After autoclaving, medium was maintained at 45-50ºC in constant temperature water bath.

Inoculation of medium with test organism

0.5 mL of 6-8h old test organism is transferred to petridish of 100 mm size (Sterilized in oven at 180ºC for 1h) using sterile micropipette. Hi-sensitivity test agar medium maintained at 45-50ºC was poured and mixed properly to ensure uniform distribution of organisms with medium. Seeded plates are allowed to set at room temperature.

Preparation of agar well for fresh leaves juice

10 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made. A 100 μL crude extract (fresh leaves juice) was transferred by micropipette per well. Plates were immediately kept at 4ºC in refrigerator for 1h for the diffusion of extract and then shifted to 35 ± 0.5ºC in incubator. Zone of inhibition was measured by zone scale after 24 h of incubation.

Preparation of agar wells for different solvent extracts

5 mm borer was used to prepare wells in agar. Four wells per plate at four equidistant corners were made. A 50 μL solvent extract was transferred by micropipette per well. Plates were immediately kept at 4ºC in refrigerator for 1 h for the diffusion of extract. And then shifted to 35ºC ± 0.5ºC in incubator. Zone of inhibition was measured after 24 h of incubation.For each bacterial strain, controls were maintained in which pure solvents were used instead of the extract. The control zones were sustracted from the test zones and the resulting zone diameter is obtained (Figs. 1 to 6).

RESULTS

All the microorganisms responded differently to the various plant extracts and standard antibiotics. All the plant extracts and antibiotics tested showed some antimicrobial activity (Table 1). The fresh leaves juice was found to be active against all organisms except *Pseudomonas aerugenosa*.When we compared the activity of aqueous extract with fresh leaves juice, the fresh leaves juice is more active. The aqueous extract found to be active against *Proteus vulgaris* and *Staphylococcus aureus*.

Table 1: Results of antimicrobial activities of fresh leaves juice and extracts of *Psidium guajava* and compared with standard antibiotics

S.No.	Microorganisms	Zone of inhibition in mm										
		Leaves extracts						Standard antibiotics				
		FJ	WE	AE	ME	CE	PE	Am30	Cf30	Co25	G50	T30
1.	*Escherchia coli*	20	-	17	17	-	16	32	29	24	17	22
2.	*Proteus vulgaris*	27	11	15	15	-	16	-	23	31	20	24
3.	*Pseudomonas aerugenosa*	-	-	21	18	-	18	14	36	-	34	22
4.	*Staphylococcus aureus*	ND	12	15	13	-	13	31	23	20	16	17
5.	*Bacillus cereus*	20	-	-	-	-	-	15	27	-	23	24
6.	*Bacillus subtilis*	13	-	16	16	15	14	31	50	36	40	32
7.	*Bacillus megaterium*	12	-	11	10	-	10	29	46	24	23	33

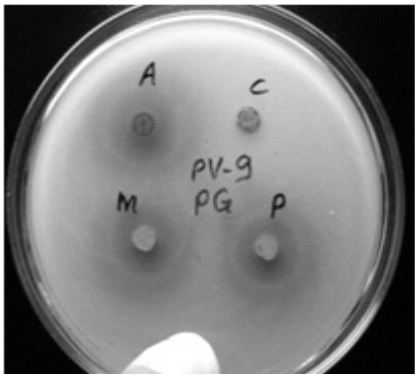

Figure 1: Activity against *Proteus vulgaris* Acetone extract (A)-15 mm Chloroform extract (C) - Methanol extract (M)-15 mm Petroleum ether extract (P)-16 mm

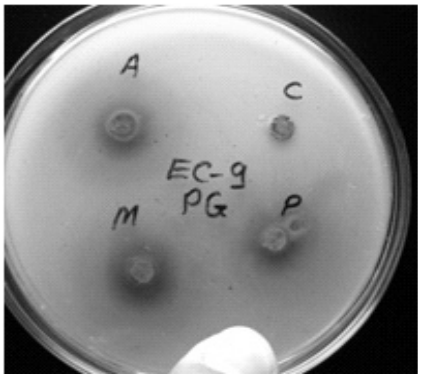

Figure 2: Activity against *Escherchia coli* Acetone extract (A)-17 mm Chloroform extract (C) - Methanol extract (M)-15 mm Petroleum ether extract (P)-16 mm

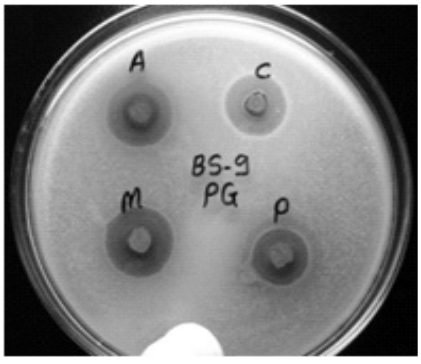

Figure 3: Activity against *Bacillus subtilis* Acetone extract (A)-16 mm Chloroform extract (C)-15 mm Methanol extract (M)-16 mm Petroleum ether extract (P)-14 mm

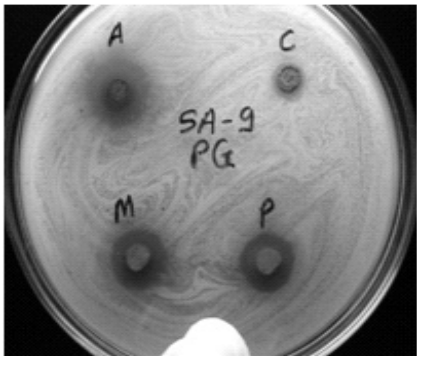

Figure 4: Activity against *Staphylococcus aureus* Acetone extract (A)-15 mmChloroform extract (C) -Methanol extract (M)-13 mm Petroleum ether extract (P)-13 mm

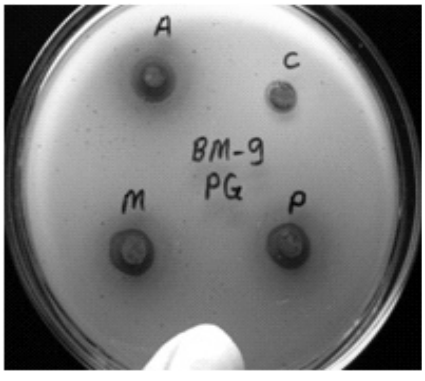

Figure 5:Activity against *Bacillus megaterium* Acetone extract (A)-11 mmChloroform extract (C) -Methanol extract (M)-10 mm Petroleum ether extract (P)-10 mm

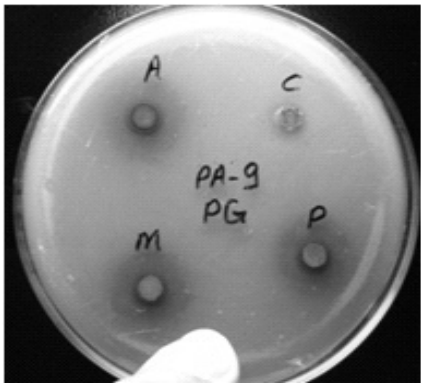

Figure 6: Activity against *Pseudomonas aeruginosa* Acetone extract (A)-21 mmChloroform extract (C) - Methanol extract (M)-18 mm Petroleum ether extract (P)-18 mm

Acetone, methanol and petroleum ether extracts are active against almost all microorganisms except *Bacillus cereus*.Chloroform extract is active against only *Bacillus subtilis* (Fig. 3). All the organisms are susceptible to Ciprofloxacin-Cf[30], Gentamicin-G[50] and Tetracycline-T[30]. *Proteus vulgaris* is found to be resistant to Amoxycilin Am[30] *Pseudomonas aerugenosa* and *Bacillus cereus* found to be resistant to Cotrimaxozole Co[25].

DISCUSSION

Successful prediction of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure.Traditional medicinal plants are used primarily water as the solvent but in our studies we found that plant extracts in organic solvent (acetone, methanol and petroleum ether) provided more consistent antimicrobial activity compared to 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.

The traditional healers use primarily water as the solvent but Suresh *et al. (*2008) found in their study the plant extracts by chloroform provided less consistent antimicrobial activity compared to those extracted by water and other solvents. In our study chloroform extract is found to be active against only *Bacillus subtilis*.It has been shown that chloroform and its impurities CH_2Cl_2 and CH_2ClBr, may react with some compounds as in the case of certain alkaloids (e.g. brucine, strychnine, and ephedrine), producing quaternary salts and other products (Phillipson and Bisset, 1972). Similarly, the presence of traces of HCl may produce decomposition, dehydration, or isomerization in other compounds (Britton *et al.,* 1991).

Out of all the solvents, water is the most important of all extraction solvents (Mukherjee, 2005). In our study fresh leaves juice showing more consistent antimicrobial activity.

It was clear that the methanol extract of selected medicinal plants exhibited high activity against the tested organisms rather than aqueous extract of those plants. Methanolic extracts of plants generally posses terpines and phenolics, which are reported by different workers as antimicrobial compounds (Manach, *et al.,* 2001; Begum *et al.,* 2002; Sanches *et al.,* 2005). Acetone, Methanol and Petroleum ether extract of *Psidium guajava* showed pronounced activity against all the tested Gram positive and Gram negative microorganisms including *Pseudomonas aeruginosa*. (Mohamed *et al.,* 1994; Kamath *et al.,* 2008 and Dey *et al.,* 2010). 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 (Jairaj *et al.,*1999; Coutino-Rodriguez *et al.,* 2001). The methanolic extract of *P. guajava* (leaves) was the only agent showing significant inhibitory (and antidiarrheal) activities.

Guava is rich in tannins, phenols, triterpins, flavonoids, essential oils, saponins, carotenoids, lectins and all those compounds together showing antimicrobial activities (Kamath *et al.,* 2008). Guava extracts of all polarity were found to be active against bacteria,indicating that more than one component may be responsible for the observed antimicrobial activity. Past research findings indicate the presence of polyphenolic compounds in guava, quercetin, avicularin and guaijaverin (Seshadri and Vasista, 1964) being the active antimicrobial components in guava leaf.

These findings support the traditional knowledge of local users about their selection of plant samples as antimicrobial agents and it is a preliminary scientific validation for the use of these plants for antibacterial activity. To promote proper conservation and sustainable use of such plant resources, awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings. The results of the present study also support the medicinal usage of the studied plants and suggest that some of the plant extracts possess compounds with antibacterial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The most active extracts can be subjected to isolation of the therapeutic antimicrobials and undergo further pharmacological evaluation.

In conclusion Acetone, Methanol and Petroleum ether extracts of the plant parts showed antibacterial activity against disease-causing organisms and this suggest that constituents of the plants could be useful in chemotherapy. From the findings of this study, the following recommendations could be made; Firstly, there is a need to further isolate the active antibacterial agent (s) and secondly, it is necessary to determine toxicity of the active constituents, their side effects and pharmacokinetics effects.

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