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IN VITRO STUDY OF ANTIBACTERIAL POTENTIAL OF ANTIBIOTIC AND *PSIDIUM GUAJAVA* PULP AGAINST *KLEBSIELLA PNEUMONIAE*

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By observing the alcoholic and aqueous extracts of *Psidium guajava* parts against human pathogen *Klebsiella pneumoniae*, it is found that the combination of extract and antibiotic chloremphenicol is more effective than antibiotic alone. The mixture of alcoholic extract and antibiotic was found more effective than the mixture of aqueous extract and antibiotic.

Keywords : Antibacterial potential; Bioassay; Chloremphenicol; *Klebsiella pneumoniae; Psidium guajava.*

Introduction

In ancient times the diseases were cured by plants without knowing the active principle or causal organism. In present system of curing the diseases microorganisms are first identified and then important drugs (antibiotic) are advised. It is commonly seen that the doctors prescribe the fruits and vegetables along with the medicines for nutrient diet. Fruits are the natural and rich source of various vitamins and amino acids etc.

In present investigation an attempt was made by using fruit and leaf extract with a broad spectrum antibiotic for curing the diseases. Aqueous and alcoholic extracts of *Psidium guajava* leaf, plup and seed were tested against the human pathogen *Klebsiella pneumoniae* that causes disease Pneumonia. The antibacterial potential of fruit plant part extracts are compared with a antibiotic chloremphenicol individually and plant extracts in combination with antibiotic.

Materials and Methods

Fresh leaves, fruit pulp and seeds of *P. guajava* were first washed with tap water and then thoroughly washed with sterilized distilled water so as to remove dust and soil particles. Hundred gm of the above plant materials were crushed in mixer grinder, each soaked in 250 ml of distilled water and absolute alcohol separately. Both mixtures were kept for 24 h at room temperature. The mixtures were filtered through muslin cloth and then they were refiltered through Whatman filter paper no.42. The alcoholic filtrate thus obtained were concentrated by evaporating leaving behind a pure extract. These pure extracts were ready for use and kept in a screw tight sterilized glass bottles.

The test organism *K. pneumoniae* in present investigation was obtained from Institute of microbial technology (IMTECH) Chandigarh 2001. Antibacterial potential of *P. guajava* plant parts were tested by microbial bioassay method[1]. Nutrient broth medium (liquid) was prepared and distributed among several test tubes. To each tube 10ml of liquid medium, 0.5 ml of 45 h old nutrient broth culture of *K. pneumoniae* and 1 ml of different filter sterilized extract was added. Inoculated medium devoid of extract was used as control 1st (+ve control) C_1 and with 0.5 ml of formaldehyde solution was used as control 2nd (-ve control) C_2 with the bacterial inoculum. One blank control 3rd C_3 was also maintained. Three replicates for each extract were used. In one set inoculated medium with 0.5 ml antibiotic devoid of extract was used as control A_1 and besides this some tubes were with inoculated medium and 1 ml of extract and 0.5 ml of antibiotic.

These tubes were incubated in B.O.D. incubator for 24 h at 37°C. After proper incubation to each incubated tube 0.5 ml of formaldehyde solution was added as a stopping agent for microbial growth. The optimal density of each set was measured

Table 1. Antibacterial potential of *Psidium guajava* on *Klebsiella pneumoniae* by tube dilution method.

Plant Part		Aqueous Extract	Alcoholic Extract	Antibiotic + Aqueous Extract	Antibiotic + Alcoholic Extract
Leaf		0.710	0.552	0.109	0.101
Pulp		0.360	0.292*	0.082	-0.072
Seed		0.539	0.475	0.062	0.042
	S. D.	0.142	0.108	0.019	0.071
	S. E.	0.081	0.062	0.010	0.040

C1 = Inoculated medium = 0.330
C2 = Inoculated medium + 0.5 ml formalehyde = 0.000
C3 = Uninoculated medium = 0.000
A1 = Inoculated medium + Antibiotic = 0.092
* = Lowest optimal density
S.D. = Standard Deviation
S.E. = Standard Error

Table 2. Composition of chemical compounds in *Psidium guajava* pulp.

Chemical compounds	Extract in ethanol	Extract in cold D. W.	Extract in hot D.W.	Extract in Acetone	Extract in Pet. ether	Extract in Ethyl acetate
Alkaloid	++	++	++	++	++	++
Flavannoid	+	+	+	+	+	+
Carbohydrates	+++	+++	+++	+++	+++	+++
Protein	+	+	+	+	+	+
Triterpenoids	+	+	+	+	+	+
Glycosides	++	++	++	++	++	++
pH	4.32	3.18	3.86	4.06	3.72	3.42
Standard pH	5.16	7.04	6.01	5.34	5.48	4.74

\+ = few ++ = moderate
+++ = good D.W. = Distilled Water

by spectrophotometer at 530 nm and compared with +ve control. In these sets, efficiency of pure extracts were compared with+ve control C_1 and A_1. For the evaluation of chemical composition and the qualitative analysis the extract was screened[2]. For the analysis of chemical compounds, extracts were prepared in ethyl alcohol, acetone, ethyl acetate, petroleum ether, hot distilled water and cold distilled water. The presence of protein was clarified by Xanthoproteic test and ninhydrin test. The presence of carbohydrates was clarified by Benedict's reagent. The presence of flavanoids was clarified by alkaline reagent test and ferric chloride solution. The presence of alkaloids was clarified by Mayer's test and Dragendroff's test. The presence of triterpenoids was clarified by Liebermann Burchardts test[3]. The pH of each extract was also noted and compared with respective solvents.

Results and Discussion

In present investigation *K. pneumoniae* was tested against different parts of *P. guajava* for its antimicrobial properties. When bacteria were treated with leaf, pulp, seed alcoholic and aqueous extract, the maximum suppression of growth of bacteria was observed in alcoholic pulp extract i.e. OD 0.292 whereas in control OD was 0.330. When pulp extract was compared with antibiotic, it showed OD of 0.092. In another set of experiment when pulp extract and antibiotic were mixed to see the effect on the growth of bacteria it was noted that alcoholic and aqueous pulp extract with antibiotic reduced the growth of bacteria in comparison with antibiotic alone. Alcoholic pulp extract and antibiotic had shown maximum inhibition of *K. pneumoniae* i.e. OD-0.072. It is concluded that if antibiotic with pulp of *P. guajava* is given to treat the *K. pneumoniae* it will give better results. After observing the better result of pulp its phytochemical analysis was screened. Carbohydrates were good, glycosides and alkaloids were moderate while flavanoides, proteins and triterpenods were few in pulp phytochemical analysis (Table 2). pH of each plant part shows that they are more acidic.

Plant extract contain protein, glycoprotein, flavones, phenols, polysaccharides and glycoalkaloids[2]. Kobiler[4] have found antifungal compounds in oil cells of Avocado fruit mesocarp. Triterpenoid phytoalaxins from peel was isolated by El-lahlou[5]. Singh[6] reported physiochemical characters of *Parkia javanica* pods and seeds.

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