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Anthelmintic and Antibacterial Activity of Psidium Guajava Leaf Extracts

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ABSTRACT

Psidium guajava leaves have long been in use for their antioxidant and anti-inflammatory properties in the Indian local medicine system. Sustained interest in the development of plant-based drugs for the treatment of many diseases is growing nowadays. Morever, to treat mild/serious diseases, people embrace traditional medicinal goods. Since the drive for plant-based antimicrobial development increased, the present study was conducted on Psidium guajava leaves. An ethyl acetate was used to prepare the leaf extract. Paper disk plate method used to test the antimicrobial activity of the extract against wound-infection-causing pathogens viz., Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. The study revealed that Psidium guajava leaves can contain active antimicrobial compounds that may impede the growth of wound infection causing in-vitro pathogens. In the present research the antimicrobial activity of Psidium guajava leaf ethyl acetate extract against human pathogenic bacteria was evaluated. The tests also suggest that scientific studies performed commonly using herbs that have conventional efficacy claims may warrant fruitful findings and methanolic and ethyl acetate extracts of Psidium guajava leaves have been investigated for their anthelmintic activity against Pheretima posthuma and Ascardia galli.

Keywords: Psidium Guajava, Anthelmintic Activity Antimicrobial Activity, Pheretima Posthuma, Pathogenic Bacteria.

I. INTRODUCTION

Since the first half of the 20th century antimicrobial drugs have been used widely in the treatment of infectious diseases caused by bacteria and fungi[1]. At the same time, by reducing deaths caused by infectious diseases, antimicrobial therapy was successful in prolonging the average life expectancy[2]. However, the growing usage and misuse of antimicrobial drugs has contributed to the production of more resistant pathogens to antimicrobials that are widely used[3]. The advent of multidrug-resistant

bacterial strains, in particular, has made treatment of bacterial diseases more complex as compared to the first half of the last century[4]. As a result, the incidence of microbial infections in many countries around the world has risen at alarming rates due to antimicrobial resistance in recent decades[5-6]. There's an urgent need for the development of new antimicrobial compounds to overcome this challenging problem[7].Guajava psidium is evergreen small tree (Myrtaceae), commonly known as guava in English, is an important food crop and medicinal plant native to South America grown in tropical and subtropical lands[8-9]. It is widely utilized worldwide as food and in folk medicine. It is cultivated commercially in India for its edible and palatable fruits[10]. A variety of good-yielding metabolites and some have been shown to have useful biological activities, primarily of phenolic compounds, flavonoids, carotenoids, terpenoids, and triterpenes[11]. Extracts and secondary metabolites of this plant possess useful pharmacological activities, especially those from leaves and fruits[12]. P. guajava is best known for its antispasmodic and antimicrobial properties in the diarrhoea and dysentery treatment. It was also widely utilized as an oral hypoglycaemic agent[13]. Several pharmacological studies have shown this plant's potential to exhibit antioxidant, hepatoprotection, anti-allergic, antimicrobial, antigenotoxic, antiplasmodial, cytototoxic, antispasmodic, cardioactive, antitussive, antidiabetic, anti-inflammatory and anti-nociceptive activity in support of its traditional uses[14-15]. However, the acute antimicrobial assessment of P. guajava leaf extracts of ethyl acetate is still not published. Hence, we attempted these studies on the leaf extracts of P. guajava grown in India in the present investigation[16]. Helminth infections remain a major constraint on livestock production in all agro-ecological zones[17]. Today, gastrointestinal parasite control key mode is based on the commercial anthelmintic. However, wide-spread increases in anthelmintic resistance, scarcity and high costs, particularly for low-income farmers in developing countries, led to the need for other alternative methods of helminth control[18]. There is considerable and growing interest in traditional herbal dewormers among other alternative methods. The aim of the study is to evaluate anthelmintic potential of P. guajava leaf extracts[19]. Microwave extraction has proved to be more effective and efficient than its conventional counterpart, the soxhlet extraction method. The Soxhlet extraction, which is a standard technique, is a continuous solvent extraction method[20-28]. Extraction systems are used to conduct routine solvent extractions of soils, sediments, sludge, polymers and plastics, pulp and paper, biological tissues, textiles and food samples. Experiments have proved that microwaves, in comparison with the soxhlet extraction, use a lesser volume of solvent and sample and perform extraction at a much faster rate[29-40]. In the discovery of effective medicines for prevention and treatment, an outbreak of coronavirus disease (COVID-19) caused by the novel extreme acute respiratory syndrome coronavirus-2 (SARS-CoV-2) poses an unprecedented obstacle. The proximity to the patient during dental care, high generation of aerosols, and the identification of SARS-CoV-2 in saliva have suggested the oral cavity as a potential reservoir for COVID-19 transmission. Mouthwashes are widely-used solutions due to their ability to reduce the number of microorganisms in the oral cavity. Given the rapid pace of scientific research and clinical data provided by the large number of people who are rapidly infected with SARS-CoV-2, clinicians need reliable evidence of good medical care for this infection, as it is simple to do in-silico analysis in the initial stage with the aid of molecular docking software with help of chemical structure of compound. It is necessary to enhance both enzymatic stability and membrane permeation in the formulating drug delivery system for protein and peptide drugs. Soon, someday, you might be making your own drugs at home. That is because researchers have adapted a 3D printer from basic, readily available medicinal active agents fed into a drug delivery system[41-52].

II. MATERIALS AND METHODS

Plant Material

Psidium guajava leaves has been collected from Karad, Maharashtra, India. Department of Botony, Yashwantrao Chavan College of Science, Karad has identified the plant and authenticated it.

Extraction Process

Shade drying was done for almost a month as to avoid chemical degradation due to sunlight. Grinding of the dried material was done, with the aid of a grinder and converted into coarse powder. Extraction was done by microwave extraction. The crude powder was defatted subjected to extraction with ethyl acetate using a microwave extraction. The excess solvent present was evaporated.

Antibacterial Assay:

Preparation and suspension of test bacteria

For the preparation of suspension of test bacteria in sterile normal saline, 18 to 24 hours old broth gram positive and gram negative developing bacteria were used. The gram positive bacteria used were Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa, the suspension turbidity of solution of the McFarland standard.

Detection of Antimicrobial activity

Paper disk plate method in which 4 mm diameter sterile paper punched by punching machine was adopted to assess P. guajava leaf-extract antibacterial activity against wound pathogens. The crude extracts were further diluted at concentrations ranging from 25µg/ml, 50 µg /ml, 75 µg/ml and 100 µg/ml with diluent. Small paper disk impregnated with each extract were placed upon the surface of an inoculated plate. After 24 hrs of incubation, the leaf-extract's antibacterial efficacy was calculated by measuring the inhibition zone[53-60].

Anthelmintic Activity:

Animals

Indian adult earthworms (Pheretima posthuma) were collected from water logged areas and Ascardia galli (nematode) worm were obtained from freshly slaughtered fowls (Gallus gallus).

Evaluation of anthelmintic activity

The anthelmintic assay was carried as per method of Ajaiyeoba et. al with minor modifications. The anthelmintic activity was evaluated on adult Indian earthworm Pheretima posthuma worm due to its anatomical and physiological resemblance with the intestinal round worms parasites of human beings. Ascardia galli (nematode) worms are easily available in slaughtered fowls and it can be used as a screening model for anthelminthic drugs as advocated earlier. Fifty milliliter of formulation containing three different concentrations, each of crude methanolic and ethyl acetate extract (10, 50,100mg/ml in distilled water) was and six worms (same type) were placed in it. This was done for both type of worms. Observation were made for the time taken to cause paralysis and death of the individual worms. Mean time for the paralysis (P)in min was noted when no movement of any sort could be observed, except when the worm was shaken vigorously; time of death (D)in min was recorded after ascertaining the worms neither moved when shaken vigorously nor when dipped in warm water (50°C). Albendazole (10mg/ml) was included as reference compound[61-69].

III. RESULTS AND DISCUSSION

Table 1. Antibacterial activity of Psidium guajava leaf extract on wound infection causing pathogens (Paper disk plate method)

Sr. no.	Wound pathogens	Diameter of zone of inhibition (mm)			
		25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
1	Escherichia coli	4	7	8	15
2	Staphylococcus aureus	6	9	11	18
3	Pseudomonas aeruginosa	8	10	14	21
4	Std (ciprofloxacin)	9	12	16	22

As shown in table 2, methanolic and ethyl acetate extract exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis (P) and death (D) with 100 mg/ml concentration. The methanolic extract of Psidium guajava caused paralysis of 10 min. and time of death of 29 min. While ethyl acetate revealed paralysis of 9 and 31 min. respectively against the earthworm Pheretima posthuma. The reference drug Albendazole showed the same at 9 and 31 minutes, respectively. Ascardia galli worms also showed sensitivity to the methanolic extract of Psidium guajava. The methanolic extract caused paralysis in 12 min, death in 31 min and the ethyl acetate extract displayed P and D in 8 and 28 min, respectively. Albendazoled id the same at 18 and 45 min.

Table 2: Anthelmintic activity of methanolic and ethyl acetate extract of Psidium guajava

Test subs	Concentration (mg/ml)	Time taken for paralysis(P) and death(D) of worms in min			
		P.posthuma		A .galli	
		P	D	P	D
Control	-	-	-	-	-
Methanolic extract	10	21.8 ± 0.45	69.0 ± 0.32	24.55 ± 0.54	42.28 ± 0.68
	50	16.05 ± 0.51	48.4 ± 0.12	16.87 ± 0.50	33.50 ± 0.31
	100	10.53 ± 0.38	29.3 ± 0.65	12.27 ± 0.49	31.68 ± 0.42
Ethyl acetate extract	10	27.12 ± 0.39	64.82 ±0.43	20.62 ± 0.41	50.09 ± 0.41
	50	18.37 ± 0.68	50.67 ±0.32	11.56 ± 0.28	35.30 ± 0.62
	100	09.45 ± 0.29	31.85 ±0.24	8.17 ± 0.53	28.14 ± 0.38
Standard (Albendazole)	10	19.48 ± 0.50	59.68 ±0.52	18.12 ± 0.45	45.74 ± 0.65

IV. CONCLUSION

Antimicrobial activity on test microorganisms was performed by paper disk plate method. Hence from observations it was revealed that successive ethyl acetate extract showed significant antimicrobial activity against most of the microorganism. The leaf extract of Psidium guajava not only demonstrated paralysis, but also caused death of worms especially at higher concentration of 100mg/ml in shorter time as compared to reference drug albendazole. Phytochemical screening of the extracts revealed the presence of alkaloids, saponins, flavonoids, triterpenes, tannins and steroids. Tannins were shown to produce anthelmintic activities chemically tannins are polyphenolic compounds. It is possible that tannins contained in the extracts of Psidium guajava produced similar effects. Reported anthelmintic effect of tannins is that they can bind to free proteins in the gastrointestinal tract of host animal or glycoprotein on the cuticle of the parasite and may cause death. Further studies are in process to identify the possible phytoconstituents responsible for anthelmintic activity.

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