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Phytochemical Characterization and Therapeutic Efficacy of *Psidium guajava* L. in Clinical Cases of Ootitis Externa

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A B S T R A C T

The present study was conducted for phytochemical characterization of leaves of *Psidium guajava* L. and also the antimicrobial potential against canine ootitis externa. Crude solvent extraction was performed with water, chloroform, ethanol and acetone. Phytochemical analysis reported presence of alkaloids, flavonoids, tannins, saponins, quinines, etc. which are responsible for the antimicrobial activity of the plant. The result was found that, the extracts of Guava leaves were found effective against the growth of test organisms. EEPG may be served as antibacterial agents and can be used in the treatment of ootitis externa condition in canines.

Introduction

Otitis externa in dogs is an acute or chronic inflammation of the external ear and the dogs with long pendulous ears are most commonly affected (Petrov *et al.*, 2013). The predisposing factors include anatomical ear canal stenosis, hair in the ear canal, pendulous ears, increased humidity, washing, foreign bodies, obstructive diseases (neoplasms) and systemic conditions (immunosuppression) (Rosser, 2004). Naturally occurring microbial agents in the ear canal are opportunists and grow whenever they found a favorable medium and are the potent cause of ootitis in canines. Therapeutic management required use of antibiotics, resistance to these drugs by microorganisms has also increased. Pharmacological industries have produced large number of new antibiotics in the last three decades but, bacteria have the genetic ability to transmit and acquire drug resistance (Gislene *et al.*, 2000).

In view of increasing resistance to existing antimicrobial agents, some research has been performed worldwide to identify herbal drugs, as they are very important sources for

discovering some new agents for treating various ailments related to bacterial infections (Pan *et al.,* 2012). Medicinal plant possesses many bioactive compounds including phenolic and polyphenolic compounds which exhibit antimicrobial activities and can be of great significance in therapeutic treatments (Hara-Kudo *et al.,* 2004). *Psidium guajava* is a phytotherapic plant used in folk medicine and is believed to have active components that helps in treatment and management of various diseases (Kenneth *et al.,* 2017). Hence, studies pertaining to the use of medicinal plants as therapeutic agents should be emphasized, especially those related to antimicrobial potential. The objective of this research was phytochemical characterization and evaluation of therapeutic potential of *Psidium guajava* L. extracts on causative agents of ootitis externa in canines.

Materials and Methods

Animals and Samples

The present study included 113 dogs of both genders from different breeds and from 6 months to 12 years old, all with clinical signs specific for otitis externa. Patients were presented with unilateral or bilateral ear drop, pain when palpated, head shaking, pruritus, erythema and swelling of ear canal with increased amount of cerumen. A total of 187 samples of ear secretion were collected using sterile cotton swabs. The study was performed all-round the season from Janury, 2017 to December, 2017.

Extract preparation

Fresh and healthy leaves of *P. guajava L*. after proper authentication, were collected at locations of Durg district of Chhattisgarh state washed and allowed to shade dry for 7 days. The leaves were then powdered finely using blending machine. The powdered samples were extracted for by using water, chloroform, ethanol and acetone in order to extract polar and nonpolar plant ingredients. The extract yields were stored in small bottles at 5°C and their yield percentages were calculated using the following formula:

Extract yield% = Weight of extracted plant residue/ weight of plant raw sample×100.

Phytochemical characterization

The plant extracts prepared in different solvents were screened for the presence of alkaloids, flavonoids, glycosides, tannins, saponin, terpinoids quinones by using the following standard test.

One ml of Mayer's reagent was added to the herbal extracts. The formation of creamy colour precipitate indicated the presence of alkaloids. A test for flavonoids includes the addition of little lead acetate and 5 drops of conc. hydrochloric acid in the extracts, appearance of crimson red colour indicated the presence. In the herbal extract 0.4 ml glacial acetic acid mixed with 3 drops 5% W/V ferric chloride was added. 0.5 ml of conc. Sulphuric acid was added by the side of test tube. Appearance of bluish green in upper layer indicates the presence of glycosides. Plant extracts treated with 1mL of 5% ferric chloride. The presence of tannin was confirmed by the formation of greenish black precipitate. Plant extracts after addition of distilled water were shaken vigorously. The formation of profuse foam layer indicated the presence of saponins. Extract treated with 1mL of concentrated sulfuric acid and formation of red colour confirmed the presence of quinones.

Microbiological tests

The collected ootic inoculum was inoculated into the nutrient broth and incubated at 37^0C

for 24-48 hours. The growth of enriched inoculums from all the samples were streaked on primary media like nutrient agar and blood agar, and incubated at 37^0C for 24-48 hours. After incubation, isolates were identified according to Standard microbiological technique (Quinn *et al.,* 1999).

Antibacterial activity of plants extract

Muller Hinton agar and Nutrient broth was used as the media for the culturing of bacterial strains. The plant extracts prepared in different solvents (100 mg/ml) were placed on 6mm sterile discs of Whatman filter paper no.1. Anti- bacterial activities of plant extracts were tested separately using disc diffusion method (Bauer *et al.,* 1966). Sterile filter paper discs loaded with plant extract were placed on the top of Mueller-Hilton agar plates inoculated with bacterial suspension. The compounds present in the extract were allowed to diffuse for 10 min and the plates were incubated at 37°C for 24 hours for bacteria. At the end of incubation, the presence of inhibition zones considered as indication for antibacterial activity were measured by Venire caliper and recorded with the different extracts.

Statistical analysis

The mean and standard error of the observed values were calculated and the data was analyzed by ANOVA method using one way analysis of variance followed by Duncan's multiple range test as per described by Snedecor and Cochran (1994).

Results and Discussion

Common etiological agents in ootits externa

In the present study high prevalence of *Staphylococcus spp.* (62%) was reported, *Pseudomonas spp.* (28.56%) was the second most predominant bacteria. Followed by *Streptococcus* (11.46%), *E. coli* (7.33%) and *Proteus* (3.96%) The findings of present study are in accordance with findings of Petrov *et al.,* (2013)

Extract yield

The plant extracts yield obtained by using different solvents is presented in table 1. The percent yield is variable due to active ingredient extracted with the solvent. The maximum yield was observed by using non-polar solvent ethanol followed by water chloroform and acetone. Arya *et al.,* (2012) also reported maximum yield with ethanol extract and minimum with petroleum ether extract.

Phytochemical characterization

Results revealed the presence of active constituents like alkaloids, flavonoids, glycosides, tannins, saponins and quinones in all the extracts prepared from leaves of *Psidium guajava* (Table 2). Farhana *et al.,* (2017) recorded the presence of alkaloids in hydroalcoholic extract of *P. guajava*. Water is a common solvent used in the extraction process, but ethanol exhibits higher polarity than water, so it can solubilize polar compounds more efficiently than extract prepared using water as solvent. Kim *et al.,* (2007) also correlated the extraction efficiency of the bio-active ingredients with the polarity of solvents. The presence of these secondary metabolites in plants produces some biological activity in man and animals and it is responsible for their uses as herbs in primary health care (Koona and Budida, 2011). Hill (1985) reported that the medical value of the plants lies in some chemical substances that produce a definite physiological action. The most important of these bioactive constituents of plant are alkaloids, tannins, flavonoids and phenolic compounds. Extract of Guava leaves has anti-inflammatory and antimicrobial

activities. These effects are probably due to the presence of polyphenolic compounds (Barbalho *et al.,* 2012).

Antimicrobial sensitivity of plant extract

Antimicrobial screening test of crude leaves extracts of *Psidium guajava* prepared in water, chloroform, ethanol and acetone was conducted. The results revealed that all plant extracts were potentially effective in suppressing microbial growth with variable potency depending upon the solvent used in extraction. Antibacterial activity as evidenced by their zones of inhibition against the growth of *Staphylococcus spp., Pseudomonas spp., Streptococcus spp., E. coli and Proteus spp.* The result was found that ethanol extract of *Psidium guajava* exhibited maximum activity and chloroform and acetone extract presented similar type of activity against the bacterial spp isolated from canine ootitis extrena. All the extracts showed almost similar activities against *Proteus spp. E. coli* was least affected with chloroform extract as compared to all the studied extracts. This concluded that among all the studied microbes, ethanolic extract of guajava leaves was found effective and can be used in the treatment of ootitis externa in canines. Similar observations were recorded by Farhana *et al.,* (2017). Ismail *et al.,* (2012) reported that presence of antimicrobial activity of guava leaf extracts has been found to be due to specific metabolites, such as alkaloids, tannins, glycosides, and flavonoids.

Gill and Holley, (2006) suggested that antimicrobial bioactive ingredients of the plant extracts (alkaloid, saponin, tannin and phenolic compounds) interact with enzymes and proteins present in bacterial cell membrane causes its disruption and alter flux of protons towards cell exterior which may induce induces cell death or inhibit enzymatic activities required for amino acids biosynthesis. Tiwari *et al.,* (2009) attributed the antimicrobial effect of herbal extracts which leads to react with microbial cell membrane disturb the structures and alter the permeability.

Table.1 Yield of extract using different solvents

Extracts in different solvents	Yield (% w/w)
Hydro extract of *Psidium guajava* (HEPG)	5.98
Chloroform extract of *Psidium guajava* (HEPG)	3.26
Ethanol extract of *Psidium guajava* (HEPG)	6.42
Acetone extract of *Psidium guajava* (HEPG)	5.22.

Table.2 Phytochemcial ingredients in extracts prepared form *Psidium guajava* leaves

Extracts	Alkaloids,	Flavonoids,	Glycosides,	Tannins,	Saponin,	Quinones
WEPG	-	+	+	++	+	+
CEPG	+	+	+	+	-	-
EEPG	++	++	++	++	-	+
AEPG	+	+	++	+	-	+

Table.3 Antimicrobial screening test of leaves crude extracts of *Psidium guajava* (100 mg/ml) against some bacterial strains canine ootitis externa

Bacterial strains	Zone of inhibition in millimeter (mm)			
	Aqueous	Chloroform	Ethanol	Acetone
Staphylococcus	12.46 ± 1.23^{bA}	10.49 ± 0.26^{bA}	15.63 ± 0.86^{aA}	10.23 ± 0.74^{bA}
Pseudomonas	10.36 ± 1.36^{bA}	6.51 ± 0.44^{bB}	12.63 ± 0.38^{aB}	7.43 ± 0.86^{bB}
Streptococcus	8.57 ± 1.01^{bB}	7.41 ± 0.28^{bB}	10.51 ± 0.29^{aB}	7.32 ± 4.83^{bB}
E. coli	10.42 ± 1.41^{bA}	7.27 ± 0.33^{bB}	12.65 ± 0.62^{aB}	8.36 ± 0.38^{bA}
Proteus	6.74 ± 1.22^{bB}	7.46 ± 0.64^{bB}	7.72 ± 0.47^{bC}	7.69 ± 0.53^{bB}

Mean± SEM with different superscripts (small alphabets) with in rows and capital in column differ significantly at $p<0.05$.

Therapeutic efficacy of extract against otitis externa

As per the result of the antibiotic sensitivity of *Psidium guajava* L, ethanolic extract (100mg/ml) was advised for tropical application thrice daily on the lesions of ootitis externa patients. Clinical recoveries in otitis externa affected canine patients were recorded on the basis of improvement in clinical signs. The crude extract was found effective and animals recovered completely after 7 days of treatment.

Ethanolic extracts contains maximum concentration of tannins, flavonoids, Phenolic compounds which are the important plant constituents and confer antimicrobial ability. The present study suggested that ethanol plant extract of *Psidium guajava*, which proved to be potentially effective, may replace the use of synthetic antibiotic to treat the cases of ootitis extrena in dogs. Ethanolic extracts contains maximum concentration of tannins and flavonoids Phenolic compounds are also very important plant constituents because their hydroxyl groups confer antimicrobial and other biological ability.

The phytochemicals and antimicrobial studies of *P. guajava* leaf ethanolic extracts provided scientific evidence of antibacterial activity due to presence of some useful phytochemicals, and was used successfully in the treatment of diseases (ootitis externa) caused by some bacterial pathogens such as *Streptococcus spp., Staphylococcus spp., Pseudomonas spp., E. coli,* and *Proteus Spp.* The results obtained in this study thus suggest that the identified phytochemical compounds are the bioactive constituents responsible for the antimicrobial efficacy of the leaves of the plants. Hence it could be inferred that the plant extracts could be a source for the industrial manufacture of drugs useful in the chemotherapy of some microbial infection. Further research is necessary to reveal its detailed molecular mechanism behind these phytochemical and antibacterial activities.

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