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RESEARCH ARTICLE

PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF *PHYLLODIUM PULCHELLUM* L. Desv. AN IMPORTANT MEDICINAL PLANT

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Abstract

Present study reveals the evaluation of phytochemical analysis and antibacterial activity in different solvents like Aqueous, Ethanol and Chloroform leaf of *Phyllodium pulchellum* (L.) Desv. The antibacterial screening was carried out by disc diffusion method using six strains of the test micro-organisms including *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 3160), *Streptococcus pyogenes* (MTCC 442), *Pseudomonas aeruginosa* (MTCC 424), *Klebsiella pneumonia* (MTCC 3384) and *Escherichia coli* (MTCC 443). The crude plant extracts showed broad spectrum activity against all bacteria. The highest inhibitory (IZ 3.4±0.52 cm) was observed in ethanolic leaf extract against *Staphylococcus aureus*. The results showed that the ethanol leaf extract was more potent than the chloroform extract and aqueous extract. Preliminary phytochemical screening of the leaf extract was carried out and it revealed the presence of alkaloids, flavonoids, steriods, terpinoids, tannins, saponins and phenol. Based on the result it could be inferred that *P. pulchellum* would be a reliable source for the treatment of some common diseases caused by resistant pathogens.

Introduction:-

Medicinal herb as a potential source of therapeutic aid has a significant role in health system all over the world for both humans and animals not only in the diseased condition but also as a potential material for maintaining proper health (Pathak and Das, 2013). Plants are a rich source of diverse type of medicines in different countries and produce a diverse array of bioactive molecules, the source of potential and powerful drugs (Hemraj Vashist and Anil Jindal, 2012). Thus, natural products with pharmacological or biological activities still play a very important role in medicine (Bhore *et al*., 2012). Plant extract has a potential application as natural medicine and to treat diseases as well as the microbiological safety of the human health (Subashkumar *et al*., 2013). Medicinal plants and their parts represent a rich source of antibacterial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Different extracts from traditional medicinal plants have been tested. Many reports have show the effectiveness of traditional herbs against microorganisms, as a result, plants are one of the bedrocks for modern medicine to attain new principles (Sankar kumar dey *et al*., 2010).They have been widely used as traditional treatments for numerous human diseases. In less developed countries low income people such as farmers, people of small isolate villages and native communities use herbal medicine for the treatment of common infections.

Phylldoium pulchellum (L.) Desv. is a sub shrub belong to the family Fabaceae, sub-family (Papilionaceae) commonly known as *Vellalothi* is mainly distributed in deciduous to Eastern Ghat of Tamil Nadu. This plant is used as a folk medicine. Biological activity such Anthelmintic (Muckda *et al*., 1989), Anti-Hepatofibrotic (Yu *et al*., 1999), anti-inflammatory (Sadia Noor *et al*., 2013), Anti-diabetic activity (Asolkar *et al*., 1992 and Jain, 1991) and Anti-Diarrheal (Khalilur *et al*., 2013) have been reported only for *Phyllodium pulchellum*. It is used for the treatment of ulcer, malarial, fever, cold, bone pain, swelling, diarrhea, wound, eye and liver affliction. It is necessary to

evaluate, in a scientific base, the potential use of herbal medicine for the treatment of infectious diseases produced by common pathogens. Many medicinal plants traditionally used for thousands of years are present in a group of herbal preparation of the Indian traditional health care system.

Materials and Methods:-

Plant collection and identification:-

The leaf of the plant (*Phyllodium pulchellum* (L.) Desv.) were collected from the *Jambhuthu* hamlet is situated at Boda hill of Namakkal District, Tamil Nadu. This plant was authenticated from taxonomist Botanical Survey of India (BSI) Coimbatore, Tamil Nadu, India. The voucher specimens were deposited in the herbarium, Department of Botany, National College (Autonomous), Tiruchirappalli. Dried material of plant parts was ground into fine powder in an electric blender and it was kept in airtight bottles at room temperature for further use.

Preparation of Crude extraction:-

Solvent of extraction

For solvent extraction, 10 g of air-dried powder was taken in 100 ml of organic solvent (ethanol and chloroform) in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 190-220 rpm for 24 h. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one-fourth of the original volume (Parekh *et al*., 2005) and stored at 4ºC in airtight bottles.

For aqueous extraction, 10 g of air-dried powder was added to distilled water and boiled on slow heat for 2 h. It was then filtered through 8 layers of muslin cloth and centrifuged at 5000g for 10 min. The supernatant was collected. This procedure was repeated twice. After 6 h, the supernatant collected at an interval of every 2 h, was pooled together and concentrated to make the final volume one-fourth of the original volume (Parekh *et al*., 2005). It was then autoclaved at 121°C temperature and at 15 lbs pressure and stored at 4ºC.

Preliminary Phytochemical screening:-

Phytochemical analysis:-

The three different solvents (Aqueous, Ethanol and Chloroform) prepared were taken and preliminary phytochemical analysis is done by using the standard procedure of (Brindha *et al*., 1981) to identify the presence of some secondary metabolites using the following tests.

Test for Alkaloids: Dragendorff's test: about 1 ml of leaf extract, 1 ml of Dradendorff's reagent was added and mixed. A dark orange or orange red precipitates indicate the presences of alkaloids.

Test for Flavonoids: Shinoda test: To 1ml of the extract, add 8 - 10 drops of concentrate HCl and a pinch of magnesium powder or filing. Boil for 10 to 15 minutes and cool. A red coloration indicates the presence of flavonoids.

Test for Steroids: Libermann Burchard test: To 0.5 ml of the extract, add 2ml of acetic anhydride and 2ml of concentrate H_2SO_4 along the sides of the tube. The formation of green colour indicates the presence of steroids.

Test for Cardiac Glycosides: Keller-Killani test: To 5ml of the extract is treated with 2ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of cardiac glycosides.

Test for Terpenoids: Salkowski test: To 5ml of the extract, add 2ml of chloroform and 3ml of concentrated H_2SO_4. Formation of yellow colour ring at the interface of the two liquids that turns reddish brown colour after two minutes, showed the presence of terpenoids.

Test for Tannins: Modified Prussian blue test: To 1ml of the extract, add 1ml of 0.008M potassium ferricyanide and 1ml of 0.02M $FeCl_3$ in 0.1 M HCl. Appearance of blue colour indicates the presence of tannins.

Test for Saponins: Forth test: About 2g of the powdered sample is boiled with 20ml of distilled water in a water bath and filter. 10 ml of the filtrate is mixed with 5 ml of distilled water and shake vigorously for a stable persistent

forth. The frothing is mixed with 3 drop of olive oil and shakes vigorously. The formation of emulsion for the positive result can be observed.

Test for Phenols: Liebermann's test: To 1ml of extract add 1ml of sodium nitrite, few drops of diluted sulphuric acid and 2ml of diluted NaOH. Appearance of deep red or green or blue colour indicates presence of phenol.

Test for Reducing Sugar: Fehling's test: To 1ml of the extract, 8 drops of Fehling's (A) and 5 drops of Fehling's (B) solution are added. The tubes are heated in a boiling water bath for few minutes. Observe red precipitates conforms presence of sugar in the sample.

Test for Anthroquinones: Borutrager's test: To 1 ml of the extract, add 1 ml of 10% $FeCl_3$ and 0.5 ml of concentrate HCl. Boil in a water bathe for few minutes. Filter it and the filtrate is treated with 1 ml of diethyl ether and concentrate ammonia. Appearance of pink or deep red colour indicates the presence of anthroquinones.

Source of Microorganisms:-

Three bacterial strains, including three gram-positive (*Bacillus subtilis* MTCC 441, *Staphylococcus aureus* MTCC 3160, *Streptococcus pyrogenes* MTCC 442) as well as three gram-negative (*Pseudomonas aeruginosa* MTCC 424, *Escherichia coli* MTCC 443, *Klebsiella pneumonia* MTCC 3384). These bacterial cultures were grown in nutrient broth medium, Stock cultures were maintained on a nutrient agar slant.

Antibacterial activity:-

Disc diffusion methods:-

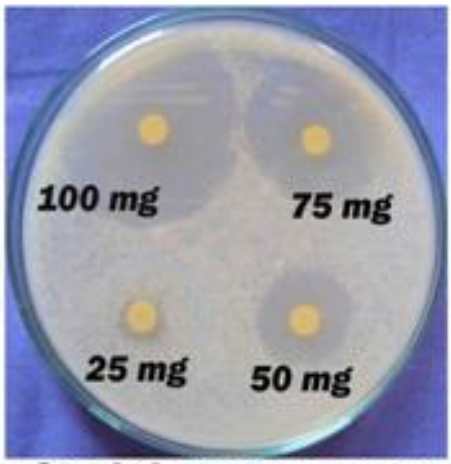
Disc diffusion method was used to test the antibacterial activity of the plant extracts against all bacteria. The essential leaf extracts used for studying their antibacterial activity. A loopful of bacterial strains were inoculated into 5 ml of nutrient broth and incubated for 6 hrs at 34ºC to get active strain. The 20 ml of sterilized agar medium was poured into each sterile Petri plates and allowed to solidify. The test bacterial cultures were evenly spread over the appropriate media by using a sterile cotton swab. The different concentrations of extracts (25mg, 50mg, 75mg and 100mg) were loaded on 6 mm sterile disc which was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37ºC for 24 hrs. The assessment of antibacterial activity was based on the measurement of zone inhibition observed around the discs (Murray *et al*., 1995). Triplicates were maintained for each extract. Inhibition zones were measured and compared with the standard reference antibiotics (Streptomycin).

Results and Discussion:-

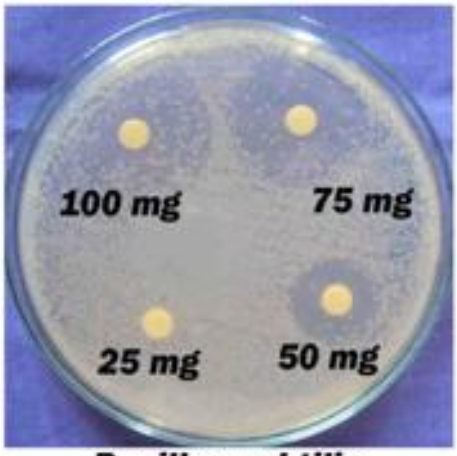
In the present study, the crude leaf extract showed excellent antibacterial activity against both tested three gram-positive and three gram-negative bacterial strains Such as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus pyrogenes*, *Pseudomonas aeruginosa*, *Klebseilla pneumonia* and *Escherichia coli* by disc diffusion methods. Aqueous, chloroform and ethanol leaf extract of *P. pulchellum* tested against various Microorganisms. Among the extracts assayed, the ethanol leaf extracts of *P. pulchellum* exhibited good activity against *Staphylococcus aureus* at 100 mg/ml (IZ 3.4±0.52 cm) was recorded as diameter zone of inhibition (Fig. 1). This was followed by Bacillus *subtilis*, *Klebseilla pneumonia*, *Pseudomonas aeruginosa*, *Streptococcus pyrogenes* and *Escherichia coli* respectively. Whereas the chloroform leaf extract showed moderate inhibition against *Staphylococcus aureus*, less inhibition was observed in *Bacillus subtilis, Klebsellia pneumonia, Streptococcus pyrogenes, Pseudomonas aeruginosa* and *Escherichia coli.* The aqueous leaf extract showed high degree of inhibition against *Streptococcus pyrogenes*. The present study revealed that the significant antibacterial activity was shown against both tested gram-positive as well as gram-negative bacterial strains. However, ethanolic extract showed the greater zone of inhibition as compared with the used standards antibiotic streptomycin against all the tested bacterial strains. The results received from the present study it was observed that the ethanolic extract of *P. pulchellum*, was the most effective as the widest inhibitory zone was noted as compared to the other solvent extract as shown in Table 1.

The results of qualitative screening of phytochemical components in leaf *P. pulchellum* revealed the presence of alkaloids, flavonoids, steriods, terpinoids, tannins, saponins and phenol presented in Table 2. From this analysis it was clear that ethanol leaf has higher extractive value, then chloroform and aqueous respectively.

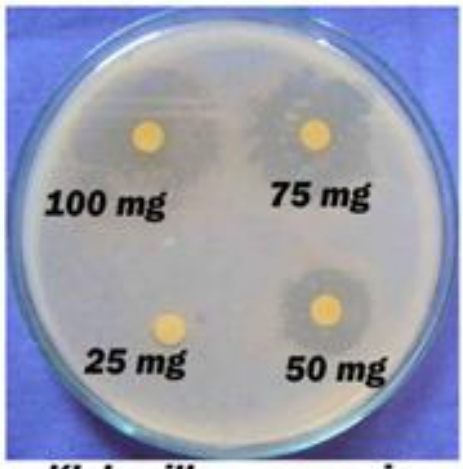
Fig. 1: Antibacterial activity of ethanolic leaf extract of against selected microbial pathogen



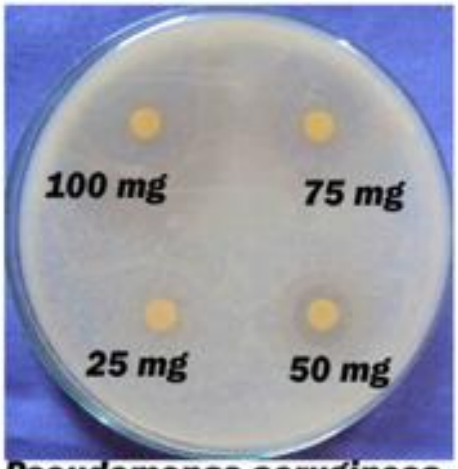
Staphylococcus aureus



Bacillus subtilis



Klebseilla pneumonia



Pseudomonas aeruginosa

Table 1: Antibacterial activity of various extracts of *Phyllodium pulchellum* of leaf against clinical pathogens:-

Test Microorganisms	Zone of inhibition (cm) Mean ± SD											
	Aqueous				Chloroform				Ethanol			
	25 mg	50 mg	75 mg	100 mg	25 mg	50 mg	75 mg	100 mg	25 mg	50 mg	75 mg	100 mg
Gram positive *Bacillus subtilis*	-	-	-	-	0.7±0.1	0.93±0.05	1.3±0.1	1.46±0.11	0.73±0.11	1.46±0.25	2.2±0.519	2.9±0.65
Staphylococcus aureus	-	-	-	-	0.73±0.11	0.9±0.1	1.13±0.11	1.66±0.11	0.76±0.15	1.93±0.50	2.76±0.55	**3.4±0.52**
Streptococcus pyrogenes	-	-	1.3±0.17	1.8±0.2	0.73±0.11	0.9±0.1	1.16±0.25	1.36±0.15	0.96±0.25	1.4±0.4	2±0.87	2.53±1.10
Gram negative *Pseudomonas aeruginosa*	-	-	-	-	-	0.76±0.05	1.03±0.15	1.23±0.25	0.83±0.15	1.73±0.30	2.06±0.50	2.6±0.72
Klebseilla pneumoniae	-	-	-	-	-	0.76±0.05	0.93±0.05	1.4±0.34	1.06±0.30	1.56±0.25	2.3±0.5	2.63±0.70
Escherichia coli	-	-	-	-	0.66±0.05	0.9±0.1	1±0.2	1.23±0.25	0.7±0.1	1.3±0.34	1.83±0.76	2.23±0.92

Table 2: Preliminary phytochemical screening of *Phyllodium pulchellum* (L.) Desv:-

S.No	Chemical compound	Solvents	Leaf	Seed
1.	Alkaloids - (Dragendorff's test)	Ethanol	+++	+++
		Chloroform	++	++
		Water	++	+
2.	Flavonoids- (Shinoda test)	Ethanol	+++	+++
		Chloroform	+++	++
		Water	++	+
3.	Steriods – Libermann Burchard test	Ethanol	+++	++
		Chloroform	++	+
		Water	+	+
4.	Cardiac Glycosides – Keller-Killani test	Ethanol	++	++
		Chloroform	+	-
		Water	+	-
5.	Terpenoids – (Salkowski test)	Ethanol	+++	++
		Chloroform	++	++
		Water	+	+
6.	Tannin – (Modified Prussian blue test)	Ethanol	+++	+++
		Chloroform	++	+
		Water	+	+
7.	Saponins – (Froth test)	Ethanol	+++	+++
		Chloroform	++	++
		Water	-	-
8.	Phenol– (Liebermann's test)	Ethanol	+++	++
		Chloroform	++	++
		Water	+	-
9.	Reducing Sugar – (Fehling's test)	Ethanol	++	++
		Chloroform	++	+
		Water	+	+
10.	Anthroquinones– (Borutrager's)	Ethanol	++	++
		Chloroform	+	-
		Water	-	-

(+++) – Strong, (++) – Moderate, (+) – Low & (-) – Absent

Conclusion:-

In the above study, the crude leaf extracts of *P. pulchellum* showed remarkable antibacterial activity against tested three gram-positive (*Bacillus subtilis, Staphylococcus aureus, Streptococcus pyrogenes*) and three gram-negative (*Pseudomonas aeruginosa, Klebseilla pneumonia, Escherichia coli*) bacterial strains. The data revealed that the ethanolic extract of the leaf showed better antibacterial activity as compared to the other two solvent extracts, which is attributed to the presence of some active components in the leaf extracts. *Phyllodium pulchellum* has been used for the anti inflammatory, anti diarrheal and anti diabetic treatments by the rural population in its growth areas in India and other country. It is one of the common ingredients of many ayurvedic medicines. Plants have become a valuable source of medicinal agents which are used for the treatment of various diseases. Therefore the future studies should be aimed to promote this plant to be used as one of the best medicinal plant is controlling pathogenic bacteria. Much attention has been paid towards plant based products which are extracted and isolated from plants.

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