ISSN 2250-3153

Antibacterial And Phytochemical Evaluation of *Pergularia daemia* from Nagapattinam Region.

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Abstract- The present study deals with the phytochemical examination and therapeutic importance of *Pergularia daemia* .The plant *Pergularia daemia* has been traditionally used as laxative ,antipyretic ,expectorant and also used to treat infantile diarrhea and malarial fever.This study involves the preliminary phytochemical screening followed by antibactertial activity. The phytochemicals such as Tannins ,Saponins ,Flavonoids ,Quinones, Phenols and Alkaloids,Steroids,Glycosides were present in the hydroalcohol of *Pergularia daemia* leaf extracts was higher amount than the other solvent extracts.In the Antimicrobial activity both gram

Index Terms- Phytochemical, Antipyretic, Antibactertial activity, *Pergularia daemia* ,MIC.

I. Introduction

The medicinal plants are useful for healing as well as for curing human diseases because of the presence of the phytochemical constitutents(Nostro et al.,2000).They are grouped as alkaloids, glycosides ,steroids, coumarins, flavonoids and essential oils.Over 50% of all modern clinical drugs are of natural origin(Cordell. G, 1995). Research in medicinal plants has gained a renewed focus recently. The prime reason is that other system of medicine although effective and it has a number of side effects that often lead to serious complications. Plant based system of medicine being natural does not pose serious problems. *Pergularia daemia* also called as *Pergularia extensa* or *Daemia extensa*, belongs to milky weed family Asclepiadaceae. Generally, the family Asclepiadaceae includes more than 2000 species classified under 280 genera are distributed world wide in the tropical and subtropical regions(Pankaj et al.,2003).

ASCLEPIADACEAE is a large genus of shrubs, and *Pergularia daemia* (forsk) chiov belongs to this family. Asclepiadaceae is foetid smelling Laticiferous twiner found in the plains though out the hot pasts of India, ascending to an altitude of 1000 meters in Himalayas (Anonymous,1997). *Pergularia daemia* asclepiadacae (Khare. C.P, 2007) known as *Pergularia* in English, Veliparuthi in Tamil, Uttaravaruni in Sanskrit and Utranajutuka in Hindi belongs to the *Pergularia* Species is a perennial twining herb and is widely distributed in the tropical and subtropical regions of Asia and South Africa and have multiple applications in different folk medicines.

Traditionally the plant *Pergularia daemia* is used as antihelmintic, Laxative , antipyretic , expectorant and also used to treat infantile diarrhoea and malarial intermittent fevers(Kirtikar. K.R, Basu.B.D,1999; Nadkarani. A.K,1976) Anonymous,1995). Latex of this plant is used for toothache (Hebbar et al 2004). Stem barks are used for cold&fever (Dokosi .O.B, 1998; Bruce .T.B.F, 1998&2000). Aerial parts of this plant have been reported for the various pharmacological activities like hepatoprotectivity (Suresh kumar et al.,2006), antifertility(Golam Sadik et al.,2001), antidiabetic (Wahi et al 2002) analgesic, antipyretic and anti inflammatory . The phytochemical investigation shows the presences of cardenolides, alkaloids and saponins(Sathish et al.,1998). The plant was found to contain various triterpenes and steroidal compounds(Anjaneyulu et al.,1998).

Many antibacterial agents are available in the Market, which are developed by the scientists. But still the microbes are challenging the scientist by developing the resistance to the presently available drugs. Plants are known to produce a variety of compounds to protect themselves against a Variety of pathogens and therefore Considered as potential source for different classes of antimicrobial Substances(Micheal.J, Pelezec, Chan. E.C.S, 1998). So, the present investigation has been carried out to shed light on the preliminary phytochemical Screening and to study the antibacterial activity of *Pergularia daemia* leaf extracts.

II. Materials and Methods

A. **Collection of Plant Sample**: The fully mature *Pergularia daemia* leaves were collected in August – September 2012 from south poigainallur village in Nagapattinam district of Tamilnadu, India and the Plant was taxonomically identified by Dr.P.Jayaraman, Plant anatomy research centre, Chennai, Tamilnadu, India. The Voucher Specimen of *Pergularia daemia was (PARC/2013/2118).*

B. Processing of plant sample: The leaves of *Pergularia daemia* are properly washed in tap water and then rinsed in distilled water. The rinsed leaves are shade dried and powdered using electrical blender. The powder was stored in air tight glass containers, protected from Sunlight until required for analysis .

Solvent extraction:

About 10 gm of powdered plant material was soaked separately in 100ml of methanol, ethanol, hydro alcohol (1:1) , ethylacetate, chloroform for 3-4 days at room temperature in dark condition. The extracts were filtered by using what man filter paper. The filtrate was concentrated to dryness under reduced pressure at 40 ^{0} C using a rotary evaporator and stored at 4° C for

further use. Each extracts was resuspended in the respective solvent and used for the qualitative and quantitative analysis of phytochemicals. For the antimicrobial testing the extracts were reconstituted to a specific concentration in dimethyl sulphoxide (DMSO).

Aqueous extraction:

10 gm of dried powdered plant leaves was soaked in 100ml of distilled water for 3-4 days at room temperature in dark condition. Then the water extract was filtered through filtered through filter paper and the filtrate was used for the phytochemical analysis.

C. Phytochemical Analysis:

The tests were done to find the presence of the active chemical Constituents such as alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins by the following Procedures.

Alkaloids: Alkaloids are basic nitrogenous compounds with definite physiological and pharmacological activity. Alkaloid solution produce white yellowish precipitate when a few drops of Mayer's reagents are added (Siddiqui et al.,1997). Most alkaloids are precipitated from neutral or slightly acidic solution by Mayer's reagent(Evans, W.C, Trease,2002). The alcoholic extract was evaporated to dryness and the residue was heated on a boiling water bath with 2% hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of mayer's reagents. The sample were then observed for the presence of turbidity or yellow precipitation.

Steroids: 1ml of the plant extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids(Gibbs ,1974).

Terpenoids: To 2ml of the plant extract was added to 2ml of acetic anhydride and concentrated H_2SO_4 . The formation of blue green ring indicate the presence of terpenoids(Ayoola et al., 2008).

Tannins: 2ml of extract was added to few drops of 1% lead acetate , and the yellowish precipitate indicated the presence of tannins(Treare, G.E, and Evans W.C ,1985).

Saponins: 5ml of Extract was mixed with 20ml of distilled water and then agitated in a graduated cyclinder for 15 minutes. Formation of foam indicates the presence of Saponins(Kumar et al.,2009).

Anthocyanins: 2ml of extract is added to 2ml of 2 N Hcl and ammonia. The appearance of pink-red which turns to blue-violet indicates the presence of anthocyanins (Paris. R and Moyse. H 1969).

Coumarins: 3ml of 10% NaOH was added to 2ml of extract and the formation of yellow colour indicates the presence of coumarins(Rizk. A.M,1982).

Flavonoids : 1 gm of the powdered sample was boiled with 10 ml of distilled water for 5 minutes and filtered while hot and few drops of 20% sodium hydroxide solution was added to 1ml of the cooled filtrate .A change to yellow colour which on addition of acid changed to colourless solution depicted the presence of flavonoids (Sofawora. A,1993; SomoLenski. S.J,1974; Harbone. J.B,1973).

Cardiac glycosides : 5 ml of extract was treated with 2ml of glacial acetic acid containing one drop of ferric chloride Solution. Then 1ml of concentrated sulphuric acid was added . A brown ring at the interface indicated the deoxy sugar characteristics of cardenolides. A Violet ring may appear below the ring while in the acetic acid layer, a green ring may be formed (Sofawora. A,1993; SomoLenski. S.J,1974; Harbone. J.B,1973).

Glycosides: Glycosides are compounds which upon hydrolysis give rise to one or more sugars(glycones) and a compound which is not a sugar (aglycone or genine).To 1 ml of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid was added and observed for a reddish brown colour at the junction of 2 layers and the bluish green colour in the upper layer) (Siddiqui, A.A et al.1997).

Phenols & Tannins : Crude extract was mixed with 2ml of 2% $Fecl_3$ solution. A blue green or black colour indicated the presence of phenols & tannins(26 Sofawora, 1993; Trease. G.E,1989; Harborne. J.B,1974).

Quinones : Dilute sodium hydroxide was added to the 1ml of crude extract and the blue green or red coloration indicates the presence of quinones.(Sofawora, 1993; A, Harbone. J.B,1973; Trease G.E,1989).

Betacyanin : About 2ml of extract was added with 1ml of 2N NaOH and heated for 5min. The Formation of bluish green colour indicates the presence of antho cyanin and Formation of yellow colour indicates the presence of beta cyanins(Yadav et al.2011).

D. Bacterial Cultures:

Gram Positive bacterial cultures like *Staphylococcus aureus*, *Bacillussubtilis*, *Staphylococuspyogenes* and gram negative Organisms like *Klebsiella pneumoniae*, *Aeromonas hydrophila*, *Escherichia coli, Pseudomonas aeruginosa*, *Vibrio Harveyi* are used as test organisms. All the test strains were maintained on nutrient agar and were sub cultured once in every two weeks.

Antibacterial assay:

Antibacterial activity of the plant extract was determined using a Kirby- Bauer disk diffusion method(Bauer et al.,1966). Briefly, 100 µl of the test bacteria were grown in 10ml of fresh media until they reached a count of approximately 10^8 cells of bacteria . And then 100µl of microbial suspension was spread on to the Muller Hinton Agar(MHA) Plates and sterile wells were made with the help of sterile cork borer.The hydro alcohol extract of *Pergularia daemia* (250, 500, 750 µg/ml) were added to the wells in aseptic conditions. The above Plates were incubated at 37^0C for 24 hours, then the diameters of the inhibition zones were measured.Each antibacterial assay was performed in triplicate & Mean Values were reported. Standard antibiotic, cipiofloxacin (10µg/well) served as positive controls for antimicrobial activity.

E.Minimum inhibitory concentration (MIC):

The minimum inhibitory concentration(MIC) of the extracts was determined by broth dilution method. The decreased concentrations of the extract were prepared (3000-250µg/ml).The extracts were weighed and reconstituted appropriately in sterile distilled water.In each tubes containing 8ml of sterile Mueller Hinton broth,1ml of the different concentration of extract,1ml overnight broth culture of the test organisms were introduced. The tubes were rolled between the palms for even mixing and incubated at $37\ ^0$ C for 24 h.Turbid tubes after incubation indicates negative and the least extract concentrations where

ISSN 2250-3153

clarity in the medium is visible to the naked eyes, determined the MIC of the extracts(Akharaiyi et al.,2011).

III. II. Results & Discussion

The preliminary phytochemical analysis of different solvents extracts of *Pergularia daemia* revealed the presence of alkaloids, flavonoids, tannins, phenolic compounds, Quinones, steroids, terpenoids and saponins as illustrated in table- 1.

The results of antimicrobial activities of 3 different concentration of (250, 500,750µg/ml) hydro alcohol (1:1) extracts of *Pergularia daemia* showed wide spectrum of activity against test organisms namely *Staphylococcus aureus, Bacillus substilis*, *Staphylococcus pyogenes*(gram positive), *E.coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, *Aeromonas hydrophila*, *Vibrio harveyi*(gram negative)(Table-2,3) .

Table-2 summarised the(gram positive) microbial growth inhibition of hydro alcohol extract of *Pergularia daemia*, The lower concentrations (250,500µg/ml) of hydro alcohol extracts does not shows any inhibitory activity on both of the bacterial strains. The results shows that the hydro alcohol extract with increased concentration (750 µg/ml) has increased inhibitory activity against *Pseudomonas aeruginosa*, *E.coli*, *Vibrio harveyi*, *Aeromanas hydrophila*, *klebsiella Pneumoniae* (ie) 12.5, 12.3, 12, 10.2, 8.2 mm.Similarly table-3 shows that (Gram negative) microbial growth inhibition of hydro alcohol extracts of *Pergularia daemia*. The Maximam zone of inhibition was observed in 750 µg/ml of plant extracts against *Bacillus substilus* and *Staphylococcus aureus*(ie) 10.2, 8.4mm. But the *Staphylococcus pyrogenes* does not show any inhibitory activity against the 3 different concentration of hydro alcohol extract of *Pergularia daemia*. The zone of inhibition produced by ciprofloxacin against gram positive and negative organism was range from 22-26.3 mm.

The MIC of hydro alcohol extracts of *Pergularia daemia* was determined by NCCL & two fold serial dilution broth method. Table-4,5,6 shows the extract of *Pergularia daemia* inhibited the growth of the *Klebsiella pneumonia*, *Aeromonas hydrophila*, *Staphyto coccus aureus*, *Staphylo coccues pyrogenes* at the concentration of 1500 µg/ml. similarly MIC of hydro alcohol extract of *Pergularia deamia* inhibited the growth of the *E.coli, Pseudomonas aeruginosa*, *Vibrioharveyi*, *Bacilles subtilis* was found to be 750 µg/ml.

Anthocyanins,glycosides,coumarins,saponins,steroids and triterpenoids were observed in a majority of species whose presence may attributed to the medicinal properties of plants(Senthilkumar. M, Gurumoorthy et al.,2011).The *Pergularia daemia* contains alkaloids ,tannins and flavonoids,cardiac glycoside and terpenes. The antimicrobial activity is probably due to the membrane disruption by terpenes and their activity might be due to their ability to form complex with extra cellular,soluble proteins and bacterial cell wall and disrupt microbial membrane (Deepika thenmozhi et al.,2011).
Several benzoquinones, naphthoquinones and athraquinones have also shown antibacterial and antiviral activities of *Pergularia daemia* (Savarimuthu ignacimuthi et al.2009). Plant based antimicrobials have enormous therapeutic potential as they serve the purpose with lesser side effect that are often associated with synthetic antimicrobials(Iwu.M.W,Duncan.A.R,Okunji. C.O,1999).

The overall results focuses that plant extracts may be potent bacteriostatic/ bactericidal agents against bacterial strains. The results from this investigation indicates that the medicinal plants extracts offer significant potential for the development of noval antibacterial therapies and the treatments of several disease caused by microorganisms.

IV. Conclusion

From the present experiment the hydroalcohol extracts of *Pergularia daemia* has antibacterial activity due to the presence of active constituents. However further research will be needed for identification of the bioactive compounds of the plant which are responsible for the pharmacological action against the disease causing microorganisms.

Table I. Phytochemical analysis

S.No	Phytochemicals	Aqueous extract	Ethyl acetate extract	Ethanol extract	Hydro alcohol extract (1:1)	Methanol extract	Chloroform Extract	Petroleum ether extract
1.	Tannins	++	+	+++	+++	+	++	-
2.	Saponins	-	-	++	++	+	++	-
3.	Flavonoids	++	-	++	+++	-	-	-
4.	Quinones	++	-	+++	+++	+	-	+
5.	Betacyanins	++	-	++	++	+	+	-
6.	Anthocyanins	-	-	-	-	-	-	-
7.	Steroids	++	-	+++	++	+	-	+
8.	Alkaloids	-	+	+	+	+	+	-
9.	Glycosides	-	-	+	+	+	-	-
10.	Terpenoids	+	-	++	++	+	-	+
11.	Cardiacglycosides	-	-	-	-	-	-	-
12.	Phenols	+	+	++	++	+	-	-
13.	Coumarins	++	-	++	+	-	-	-

Where as:+++:Strongly present,++:Mildly present

,+ Present and – Absent.

Table II. Antibacterial activity (gram positive organisms)

S.NO	Organisms	Zone of Inhibition(mm)			
		Ciprofloxacin (standard)	Hydroalcohol extracts		
			250µg	500µg	750µg
1.	*Staphylococcus aureus*	23.4±0.4	NA	NA	8.4±0.4
2.	*Bacillus subtilis*	22±0.1	NA	NA	10.2±0.3
3.	*Staphylococcus pyogenes*	25.3±0.3	NA	NA	NA

The values are expressed as mean± SD of three samples(n=3).

Table III. Antibacterial activity (gram negative organisms)

S.NO	Organisms	Zone of Inhibition(mm)			
		Ciprofloxacin (standard)	Hydroalcohol extracts		
			250µg	500µg	750µg
1.	*Klebsiell pneumoniae*	24±0.3	NA	NA	8.2±0.25
2.	*Aeromonashydrophila*	25.2±0.25	NA	NA	10.2±0.25
3.	*E.coli*	25.4±0.36	NA	NA	12.3±0.30
4.	*Pseudomonas aeruginosa*	26.3±	NA	NA	12.5±0.25
5.	*Vibrio harveyi*	24.1±	NA	NA	12±0.20

The values are expressed as mean± SD of three samples(n=3).

Table IV. Minimum Inhibitory Concentration(µg/ml)

Concentration/Organisms	*Klebsiell pneumoniae*	*Aeromonashydrophila*	*E.coli*
3000µg	0.52±0.02	0.44±0.03	0.54±0.04
1500 µg	0.30±0.02	0.31±1.4	0.57±0.03
750 µg	0.44±0.03	1.4±0.25	0.5±0.02
500 µg	1.1±0.02	1.24±0.03	0.62±0.02
250 µg	1.4±0.02	1.28±0.03	1.3±0.3
Negative control	No growth	No growth	No growth
Control	1.2±0.21	1.13±0.25	1.4±0.3

The values are expressed as mean± SD of three samples(n=3)

Table V. Minimum Inhibitory Concentration(µg/ml)

Concentration/Organisms	*Pseudomonas aeruginosa*	*Vibrio harveyi*
3000µg	0.47±0.02	0.45±0.02
1500 µg	0.34±0.03	0.33±0.03
750 µg	0.27±0.02	0.25±0.02
500 µg	0.57±0.02	1.3±0.25
250 µg	0.76±0.01	1.4±0.25
Negative control	No growth	No growth
Control	1.7±0.5	1.2±0.1

The values are expressed as mean± SD of three samples(n=3).

Table IV. Minimum Inhibitory Concentration(µg/ml)

Concentration/Organisms	*Staphylococcus aureus*	*Bacillus subtilis*	*Staphylococcus pyogenes*
3000µg	0.45±0.01	0.47±0.03	0.44±0.03
1500 µg	0.33±0.03	0.26±0.03	0.33±0.03
750 µg	0.84±0.04	0.24±0.03	0.74±0.02
500 µg	0.96±0.02	0.25±0.03	1.1±0.02
250 µg	0.97±0.01	0.57±0.02	1.3±0.25
Negative control	No growth	No growth	No growth
Control	0.86±0.02	0.88±0.01	1.5±0.2

The values are expressed as mean± SD of three samples(n=3).

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