A COMPARATIVE STUDY ON *P. DAEMIA* AND *A.MARMELOS*

R. Nithyatharani[1], U.S. Kavitha[2]
[1]Assistant Professor, [2]PG Student
Department of Microbiology
Cauvery College for Women, Trichy, India- 620 018

Abstract: Medicinal plants are the blueprint of potential drugs. In this regard, *Pergularia daemia* and *Aegle marmelos* are among them. The aim of the present study is to determine the preliminary phytochemical screening of both the leaves of *Pergularia daemia* and *Aegle marmelos* in methanol by both qualitative and quantitative analysis. This study also aims to determine the effect of methanolic extracts on Enterobacteriaceae family. Phytoconstituents such as alkaloids, terpenoids, flavonoids, saponins, carbohydrates, and proteins were identified from both samples. The quantification of flavonoids, alkaloids and phenols were done. The extracts inhibited *Bacillus sp* followed by *Escherichia coli, Klebsiella pneumoniae* and *Proteus vulgaris*. The results suggested that *Pergularia daemia* possess more phytoconstituents compared with *Aegle marmelos*.

Key words**:** *Pergularia daemia, Aegle marmelos*, medicinal plant, phytochemical, antimicrobial activity.

I. INTRODUCTION

India is widely known as the botanical garden of the world since it is the largest producer of medicinal herbs (Shariff, *et. al.*, 2006). Medicinal plants act as an indigenous source of therapeutic value and can also be used in drug development. 80% of the population of developing countries depend on traditional medicines, mostly natural plant products, for their primary health care needs as estimated by WHO (Vines, 2004).The ability to synthesize compounds by secondary metabolites possessing antimicrobial potential makes plants an invaluable source of pharmaceutical and therapeutic products (Saet, *et. al.*, 2007). The effectiveness of plant extracts on microorganism has been studied worldwide (Ates and Erdogul, 2003).

Pergularia daemia belonging to Asclepiadaceae family is a latex perennial twinning herb. It is commonly present along the roadsides of tropical and subtropical regions (Pankaj , 2003). It is commonly known as "Veliparuthi" in tamil and "Hariknot" in English. Leaves are used to treat catarrhal infection and infantile diarrhoea, stomach ache and tetanus (Irvine *et. al*., 1952), leprosy and haemorrhoids (Thatoi *et. al*., 2008), nasobronchial disease, stomach pain, antihelmetic and expectorant (Ndukwu and Ben –Nwadibia 2005), headache, cough and chest pain (Iganacimuthu *et al*., 2008), alopecia. Bruised leaves are used against eye sores and to cure wound infections (Irvine *et. al*., 1952).

Aegle marmelos is commonly called as Bael in Hindi, Vilvam in Tamil and Bilva in Sanskrit (Chopra, *et. al.*, 1982). The biological and pharmacological activity of Aegle marmelos is used to treat various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders (Gupta and Tandon, 2004). Antioxidant, antiulcer, antidiabetic, anticancer, antihyperlipidaemic, anti-inflammatory, antimicrobial, antispermatogenic effects have also been reported (Arul, *et. al.*, 2005). Leaf is considered to be one of the highest accumulatory parts of the plant containing bioactive compounds which are synthesized as secondary metabolites (Yen, *et. al.*, 1993).

The objective of the present study to determine the preliminary phytochemical screening of both the leaves of *Pergularia daemia* and *Aegle marmelos* in methanol and to determine its antibacterial activity on Enterobacteriaceae family.

II. MATERIALS AND METHODS

2.1 Collection of Plant

The plant sample *Pergularia daemia* was collected from Tirukoilur, villupuram district. The sample *Aegle marmelos* was collected from Tiruchirapalli, Tamilnadu, India. The leaves were collected and washed thoroughly with distilled water to remove the dust particles. Then the leaves were shade dried and coarsely powdered using mechanical grinder.

2.2 Preparation of the extract

The dried powdered sample was soaked in methanol for 3 to 5 days. After 5 days, the extract was filtered using No.1 Whatman filter paper and stored in air tight container for further analysis.

2.3 Qualitative analysis of phytochemicals

Preliminary phytochemical screening was carried out by the method described by (Kokate, *et. al.,* 1986 and Harbourne, *et. al.,* 1980).

2.3.1 Test for alkaloids (Mayer's test)

To the 1ml of extract, 1 ml of Mayer's reagent (Potassium iodide solution) was added. Formation of whitish yellow or cream coloured precipitate indicates the presence of alkaloids.

2.3.2 Test for steroids (Libermann Burchard test)

To the 1ml of extract, 2ml of acetic anhydride and 2ml of concentrated sulphuric acid were added. Formation of violet to blue or green colour indicates the presence of steroids.

2.3.3 Test for terpenoids (Salkowski test)

To the 1 ml of extract, 2ml of chloroform and few drops of sulphuric acid were added. Formation of reddish brown ring indicates the presence of terpenoids.

2.3.4 Test for flavonoids (Alkaline reagent test)

To the 1 ml of extract, few drops of dilute ammonium solution and few drops of concentrated hydrochloric acid were added. A yellow colouration indicates the presence of flavonoids.

2.3.5 Test for saponins (Froth test)

To the 1 ml of extract, 5 ml of distilled water was added and shaked vigorously. Formation of froth indicates the presence of saponins.

2.3.6 Test for phenols (Lead Acetate test)

To the 1ml of extract, 1 ml of lead acetate solution was added. Formation of precipitate indicates the presence of phenols.

2.3.7 Test for tannins (Lead acetate test)

To the 1ml of extract, 1ml of lead acetate was added. A formation of white precipitate indicates the presence of tannins.

2.3.8 Test for tannins (Ferric chloride test)

To the 1ml of extract, 1ml of ferric chloride solution was added. Formation of blue, black or brownish green colour indicates the presence of tannins.

2.3.9 Test for cardiac glycosides (Keller killiani test)

To the 1ml of extract, add 5ml of distilled water and evaporate it to dryness. Then to the Sample add 2ml of glacial acetic acid containing trace amount of ferric chloride solution. Then add 1ml of concentrated sulphuric acid to the sides of the tube. Formation of brown ring underlayed with blue colour indicates presence of cardiac glycosides

2.3.10 Test for aminoacids (Ninhydrin test)

To the 1ml of sample, add 3 to 4 drops of Ninhydrin solution was added and boiled in water bath for 10 minutes. Formation of purple or blue colour indicates the presence of amino acids.

2.3.11 Test for proteins (Biuret test)

To the 1ml of extract, 1ml of 40% sodium hydroxide solution and 2 drops of 1% copper sulphate solution were added. Formation of violet colour indicates the presence of proteins.

2.3.12 Test for carbohydrates (Barfoed test)

To the 2ml of extract, 1ml of Barfoed's reagent was added and boiled in water bath for few minutes. Formation of reddish brown precipitate indicates the presence of carbohydrates.

2.3.13 Test for reducing sugars (Fehling's test)

To the 1ml of extract, equal quantities of Fehling solution A and B were added and heated. Formation of brick red precipitate indicates the presence of reducing sugars.

2.4 Quantitative estimation of phytochemicals

2.4.1 Alkaloid determination

5 gm of sample was added to 200 ml of 10% acetic acid in ethanol in a beaker. The beaker was tightly covered and allowed to stand for 4 hours. This was filtered and the extract was concentrated on a water bath to one quater of the original volume. The entire solution was precipitated by the drop wise addition of concentrated ammonium hydroxide solution. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. The residue is alkaloid, which was dried and weighed (Harbourne, *et. al.*, 1980).

2.4.2 Flavonoid determination

10 gm of sample was added to 100 ml of 80% aqueous methanol in a beaker. The whole solution was filtered through Whatman filter paper No.42 (125mm). The filtrate was then evaporated to dryness and weighed (Harbourne, *et. al.*, 1980).

2.4.3 Determination of total phenols

Few grams of sample were boiled with 50 ml of ether for the extraction of phenols for 15 minutes. To the 5ml of extract, 10 ml of distilled water, 2ml of ammonium hydroxide solution and 5ml of concentrated amyl alcohol were added. The samples were left for 30 minutes. This was measured at 505 nm (Harbourne, *et. al.*, 1980).

2.5 Isolation and Identification of pathogenic bacteria

The samples were collected aseptically and then streaked on Nutrient agar, Mannitol salt agar, EMB agar medium. The isolated organisms were identified by Gram staining.

2.6 Antimicrobial activity using Agarwell diffusion method

20 ml of sterile Muller Hinton agar was poured over sterile petriplates and allowed to set. Plates were then seeded with 24 hrs old bacterial culture using sterile swabs. For agar well diffusion method, wells were made on the plate by using cork borer. Methanolic extracts were added to the well in the concentration of 50µl, 100µl, 150µl respectively. The plates were allowed to dry for 10 minutes for the diffusion of extracts into the agar. Then the plates were incubated at 37°C for 24 hrs. After 24 hrs, the plates were examined for zone of inhibition (Murray, *et. al.*, 1995).

2.7 Antimicrobial activity using disc diffusion method

Sterile Muller Hinton agar plates were prepared as agar well diffusion method. Sterile filter paper discs impregnated with methanolic extracts of concentrations 50µl, 100µl, and 150µl were placed over the agar plates. The plates were allowed to dry for 10 minutes for the diffusion of extracts into the agar. Then the plates were incubated at 37°C for 24 hrs. After 24 hrs, the zones were examined and measured in millimeters (Murray, *et. al.*, 1995).

III. RESULTS AND DISCUSSION

The qualitative phytochemical analysis of the leaves of *Pergularia daemia* and *Aegle marmelos* are summarized in the Table 1. The quantification of important phytocompounds of the leaves of *Pergularia daemia* and *Aegle marmelos* are summarized in Table 2. Pathogens such as *Escherichia coli, Bacillus sp, Proteus vulgaris* and *Klebsiella pneumoniae were* isolated from clinical samples. The methanolic extracts possess antibacterial activity and shows effective result against enterobacteriaceae family. The results obtained by Agar well diffusion method are summarized in table 3 and table 4. The results obtained by disc diffusion method are summarized in Table 5 and table 6. The size of the zone increases as the concentration increases. The antimicrobial

activity is mainly due to the presence of terpenes which can cause disruption of cell membranes (Urzua, *et. al.*, 1998). The maximum zone of inhibition is seen in *Bacillus sp* (28mm). The minimum inhibitory effect is on *Proteus vulgaris* (15mm) in methanolic extracts of *Pergularia daemia* whereas the maximum zone seen is 21mm and minimum is 12mm in *Aegle marmelos*. Thus, it is clear that the leaf samples of *Pergularia daemia* are effective against enterobacteriaceae family.

Table 1. Results of qualitative analysis of methanolic extracts of *Pergularia daemia* and *Aegle marmelos*

TESTS	*Pergularia daemia*	*Aegle marmelos*
ALKALOID	+	-
STEROIDS	+	-
FLAVANOIDS	-	+
TERPENOIDS	+	+
SAPONINS	+	+
PHENOLS	+	-
TANNINS	+	+
CARDIAC GLYCOSIDES	+	+
AMINOACIDS	+	+
PROTEINS	+	+
CARBOHYDRATES	+	+
REDUCING SUGARS	+	+

Table 2. Results of quantitative analysis of methanolic extracts of *P. daemi* and *A. marmelos*

TESTS	*Pergularia daemia*	*Aegle marmelos*
ALKALOID	8.56 ± 0.08	1.56 ± 0.08
FLAVANOID	2.03 ± 0.02	8.83 ± 0.02
PHENOLS	16.53 ± 0.35	1.53 ± 0.35

Table 3. Results of zone of inhibition using methanol extracts of *P. daemia* by agar well diffusion method.

PATHOGENS	50µl	100µl	150µl
Bacillus sp	18mm	21mm	28mm
Escherichia coli	15mm	18mm	21mm
Proteus vulgaris	8mm	10mm	15mm
Klebsiella pneumoniae	14mm	16mm	19mm

Table 4. Results of zone of inhibition using methanol extracts of *A. marmelos* by agar well diffusion method.

PATHOGENS	50µl	100µl	150µl
Bacillus sp	16mm	19mm	21mm
Escherichia coli	13mm	17mm	18mm
Proteus vulgaris	5mm	7mm	12mm
Klebsiella pneumoniae	12mm	15mm	16mm

Table 5. Results of zone of inhibition using methanol extracts of *P. daemia* by disc diffusion method.

PATHOGENS	50µl	100µl	150µl
Bacillus sp	13mm	16mm	22mm
Escherichia coli	10mm	13mm	16mm
Proteus vulgaris	5mm	7mm	10mm
Klebsiella pneumoniae	9mm	11mm	14mm

Table 6. Results of zone of inhibition using methanol extracts of *A. marmelos* by disc diffusion method.

PATHOGENS	50µl	100µl	150µl
Bacillus sp	10mm	13mm	16mm
Escherichia coli	8mm	10mm	11mm
Proteus vulgaris	4mm	6mm	7mm
Klebsiella pneumoniae	6mm	8mm	10mm

IV. CONCLUSION

In this study, the antibacterial activity of *Pergularia daemia* and *Aegle marmelos* has been investigated. The *Pergularia daemia* shows more effect than *Aegle marmelos*. This study proves that leaves of *Pergularia daemia* are more effective against enterobacteriaceae family. Further pharmacological and Pharmacognosical investigations are being carried out to identify its medicinal profile in the field of medicine.

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