PHYTOCHEMICAL ANALYSIS OF *P. DAEMIA AND A.MARMELOS*

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Abstract: Medicinal plants are the treasure house of potential drugs. They possess anti inflammatory, anti helminthic, anti pyretic, analgesic and anti cancer properties. Diabetes mellitus is one of the most common diseases in the current years. Many medicinal plants are used since ancient times to cure diabetes. In this regard, *Pergularia daemia* and *Aegle marmelos* are among them. The aim of the present study to determine the preliminary phytochemical screening of both the leaves of *Pergularia daemia* and *Aegle marmelos* in different solvents like methanol, ethanol, chloroform, petroleum ether and aqueous by both qualitative and quantitative analysis. 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 results suggested that *Pergularia daemia* possess more phytoconstituents compared with *Aegle marmelos*.

Key words: *Pergularia daemia, Aegle marmelos*, medicinal plant, leaves, phytochemical screening.

I. INTRODUCTION

Since ancient times man has used plants in the treatment and prevention of many ailments (Ariharan and Nagendra Prasad, 2014).Today number of drugs are developed from plants which are active against a number of diseases. The main reason of using plants based drugs is due to the presence of active ingredient (Amrit pal singh, 2005). In the developed countries 25 percent of the medical drugs are based on plants and their derivatives (Samy, *et. al.*, 2008) and the use of medicinal plants is well known among the indigenous people in rural areas of many developing countries. The medicinal plant therapy is based on the empirical findings of thousands of years (Vyas, *et. al*, 2011).

Pergularia daemia is a slender, hispid, fetid smelling lactiferous herb (Doss and Anand, 2013) found in tropical and sub tropical regions. It belongs to Asclepiadaceae family which includes more than 2000 species (Kathishwaran and Mirunalini, 2010). It is popularly known as "Veliparuthi" in Tamil and "Hariknot" in English. Shoots of the plant are used commonly to treat whooping cough (Kokawaro, 1981). The latex from the leaves is used to treat veneral diseases, arthritis, muscular pain, asthma and rheumatism. The bark of the stem is used in the treatment of cold and diarrhoea in infants. It also possesses antipyretic properties and analgesic activity.

Aegle marmelos is commonly called as Bael in Hindi, Vilvam in Tamil and Bilva in Sanskrit (Chopra, *et. al.*, 1982). It belongs to the family Rutaceae (Pankaj, 2003). The leaves are used as astringent, laxative, febrifuge and expectorant (Arul, *et. al.*, 2005). The leaves are useful in opthalamia, inflammations, catarrhal fever, diabetic and asthmatic complaints. The leaves are used for the heart and brain disorders.

The aim of the present study to determine the preliminary phytochemical screening of both the leaves of *Pergularia daemia* and *Aegle marmelos* in different solvents like methanol, ethanol, chloroform, petroleum ether and aqueous by both qualitative and quantitative analysis.

II. MATERIALS AND METHODS

2.1 Collection of plant sample

The leaves of *Pergularia daemia* were collected from Tirukoilur, Villupuram, Tamilnadu, India and the leaves of *Aegle marmelos* were collected from Tiruchipalli, Tamil nadu, India.

2.2 Preparation of the extract

The collected were washed thoroughly in tap water to remove dust particles. The leaves were then dried in shade at room temperature and coarsely powdered by a mechanical grinder. 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 quarter 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).

III. RESULTS AND DISCUSSION

The qualitative phytochemical analysis of the leaves of *Pergularia daemia* and *Aegle marmelos* are summarized in the Table 1 and Table 2 respectively. The quantification of important phytocompounds of the leaves of *Pergularia daemia* and *Aegle marmelos* are summarized in Table 3 and Table 4. The methanolic extract of leaves shows the presence of high number of phytocompounds when compared with other solvents like ethanol, petroleum ether, chloroform and aqueous in both samples. Phytochemicals such as saponins, terpenoids, and alkaloids have hypoglycemic activities (Cherian and Augusti, 1995). The leaves show the presence of terpenoids and they play a major role in the treatment of intestinal disorders like diarrhoea and dysentery (Akinpelu and Onakoya, 2006). The leaves show positive result for phenols which can be act as antioxidants (Rumaisa, *et. al.,* 2013). The leaves also have flavanoids which can act as antioxidants. Phytochemicals have highest therapeutic efficiency in pharmaceutical field (Thilagavathi, *et. al.,* 2015). Earlier studies suggested that *Pergularia daemia* (Wahi, *et. al.,* 2002) and *Aegle marmelos* (Sabbu and Kuttan, 2014) possess antidiabetic activity. This study suggests that *Pergularia daemia* possess more phytocompounds compared with *Aegle marmelos*. It helps to undertake further studies on isolation and identification of specific phytocomponents for pharmacological studies.

Table 1. Qualitative phytochemical analysis of the leaves of *Pergularia daemia.*

TESTS	METHANOL	ETHANOL	PETROLEUM ETHER	CHLOROFORM	AQUEOUS
ALKALOID	+	+	-	-	+
STEROIDS	+	+	+	+	+

FLAVANOIDS	-	+	+	-	+
TERPENOIDS	+	+	+	-	+
SAPONINS	+	+	+	+	+
PHENOLS	+	+	-	+	-
TANNINS	+	+	+	-	+
CARDIAC GLYCOSIDES	+	+	+	+	+
AMINOACIDS	+	-	-	-	-
PROTEINS	+	-	+	-	-
CARBOHYDRATES	+	-	-	-	-
REDUCING SUGARS	+	+	+	+	+

Table 2. Qualitative phytochemical analysis of the leaves of *Aegle marmelos*

TESTS	**METHANOL**	**ETHANOL**	**PETROLEUM ETHER**	**CHLOROFORM**	**AQUEOUS**
ALKALOID	-	-	-	-	+
STEROIDS	-	-	-	+	+
FLAVONOIDS	+	+	-	+	+
TERPENOIDS	+	+	+	+	+
SAPONINS	+	+	+	+	+
PHENOLS	-	-	-	-	-
TANNINS	+	-	-	-	-
CARDIAC GLYCOSIDES	+	+	+	+	+
AMINOACIDS	+	+	-	+	-
PROTEINS	+	+	+	+	+
CARBOHYDRATES	+	+	+	-	+
REDUCING SUGARS	+	-	-	-	-

Table 3. Quantitative phytochemical analysis of the leaves of *Pergularia daemia*

TESTS	**METHANOL**	**ETHANOL**	**PETROLEUM ETHER**	**CHLOROFORM**	**AQUEOUS**
ALKALOID	8.56 ± 0.08	7.56 ± 1.20	1.25 ± 0.67	1.32 ± 1.0	7.45 ± 1.23

FLAVANOID	2.03 ± 0.02	3.01 ± 1.0	3.85 ± 0.04	0.09 ± 0.01	4.15 ± 0.08
PHENOLS	16.53 ± 0.35	14.25 ± 2.3	5.09 ± 0.09	9.09 ± 2.12	6.72 ± 1.32

Table 4. Quantitative phytochemical analysis of the leaves of *Aegle marmelos*

TESTS	METHANOL	ETHANOL	PETROLEUM ETHER	CHLOROFORM	AQUEOUS
ALKALOID	1.56 ± 0.08	1.02 ± 1.20	0.25 ± 0.7	0.32 ± 1.07	5.45 ± 0.23
FLAVONOID	8.83 ± 0.02	8.00 ± 1.0	1.85 ± 0.04	7.09 ± 0.01	8.75 ± 0.08
PHENOLS	1.53 ± 0.35	0.25 ± 2.3	1.09 ± 0.09	0.09 ± 2.12	0.72 ± 1.32

IV. CONCLUSION

The qualitative and quantitative analysis shows that the leaves of *Pergularia daemia* and *Aegle marmelos* contain significant bioactive components such as alkaloids, steroids, terpenoids, phenols, tannins, proteins, flavonoids and saponins. The methanolic extracts are rich in phytoconstituents when compared with other extracts. Thus, the studies reveal the presence of various phytoconstituents of the leaves of *Pergularia daemia* is far higher than the phytoconstituents of the leaves of *Aegle marmelos.* Further studies are being undertaken to isolate its phytoconstituents and to identify its medicinal properties in the field of medicine.

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