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ANTIBACTERIAL SCREENING OF *AEGLE MARMELOS, LAWSONIA INERMIS* AND *ALBIZZIA LIBBECK*

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Abstract

Three medicinal plant *Aegle marmelos, Lawsonia inermis, Albizzia libbeck* were extracted by soxhlet apparatus using petroleum ether, ethanol, chloroform and aqueous as solvent. Among those extract, the petroleum ether was considered as effective one. The extracts were subjected to preliminary phytochemical screening and the three plants with four extracts were tested against three Gram positive bacteria (*B.cereus, B.subtilis, S. aureus*) and three Gram negative bacteria (*E.coli, P.vulgaris,* and *P.aeruginosa*) by disc diffusion method. Maximum inhibition (3.8cm) was recorded in *Lawsonia inermis*. It also showed inhibitory action against all the six pathogen tested. The zone of inhibition of the extracts was compared with the standard antibiotics Streptomycin and Spectinomycin. The study suggests that the plant is promising the development of phytomedicine for antimicrobial properties.

Key words: Antibacterial activity, *Aegle marmelos, Lawsonia inermis, Albizzia libbeck*

Introduction:

World is endowed with a rich wealth of medicinal plants. Man cannot survive on this earth for long life without the plant kingdom because the plant products and their active constituents played an important role. Herbs have always been the principal form of medicine in India and presently they are becoming popular throughout the world, as people strive to stay health in the face of chronic stress and pollution, and to treat illness with medicines that work in count with the body's own defense (Perumalsamy et al., 1998). There is a widespread belief that green medicines are healthier and more harmless or safer than synthetic ones (Parvathi et al., 2003). Medicinal plants have been used to cure a number of diseases. Though the recovery is slow, the therapeutic use of medicinal plant is becoming popular because of its inability to cause side effects and antibiotic resistant microorganisms (Rawat, 2003).

Antibacterial properties of various plants parts like root stem leaves, seeds, flowers, fruits have been well documented for some of the medicinal plants for the past two decades (Levan et al., 1979). Medicinal and aromatic plants and essences are rich in antibacterial compounds could be an alternate way to combat against bacterial diseases (Abramowize, 1990; Samy et al., 1998; Meera et al., 1999). Since the 1940's, but many bacteria are now becoming resistant to them. According to Braunter and Grein (1994) natural plant products may offer a new source of antibacterial agents. In recent years antimicrobial properties of Indian medicinal plants have been increasingly reported (Aswal et al., 1996; Ahmad et al., 1998). The traditional treatment approach is of much significance, especially in India due to the endemic presence of infective gastro intestinal diseases which are the major causes of infant and adult mortality (Miranda et al., 1993).

Aegle marmelos is belongs to the family Rutaceae, commonly called as Bael (English), Vilvam (Tamil) and is found throughout India . Bael is a medium sized decidous tree bearing strong axillary thorns. Leaves with 3 or 5 leaflets. Bael leaves are extremely useful for treating diabetes, jaundice, cholera and asthma. Bael leaves are made into a poultice and used in the treatments of ophthalmic. Bael leaf poultice is applied to inflammations–with black pepper for edema, constipation, and jaundice. *Lawsonia inermis* is belongs to the family Lythracea, commonly called as Henna (English), Marudhani (Tamil) and it occurs in several parts of India, chiefly in the drier parts. The paste of leaves is largely used in Indian homes in headache, burning sensation in feet etc. The leaves also have some action against tubercular and other bacteria, and in typhoid and haemorrhagia. *Albizzia libeeck* is belongs to the family Mimoseae, commonly called as Raintree (English), Vagai (Tamil). It is found throughout India and the leaves are used for the treatment of diarrhoea, dysentery and pruritis.

Against this background information and appreciating the knowledge of medicinal plants an effect has been made in this study to evaluate the antibacterial efficacy of three selected medicinal plants and also characterizing them by screening preliminary by phytochemical analysis. The study also pertains to inculcate the subject about the utilization of natural flora as therapeutic agents.

Materials and Methods

Plant preparation

The present project work was carried out in the Department of PG Biochemistry, V.V.Vanniaperumal College for Women, Virudhunagar, with the object of screening the antibacterial activity of some medicinal plants such as *Aegle marmelos, Lawsonia inermis, Albizzia libbeck.* Their botanical identities were determined and authenticated in botany department, V.V.Vanniaperumal College for women, Virudhunagar. The plant materials chosen based on therapeutic properties and their availability in our campus. The three selected plants were thoroughly washed and then dried under shade at 25 ± 2^0C for about 10 days. The dried plant samples were ground well into a fine powder in a mixer grinder and sieved to give particle size of 50–150mm. The plant powder was stored in air sealed polythene bags at room temperature before extraction.

Extraction procedures

25g of dried plant powder was packed in a Whatmann filter paper no.1 and was extracted in a soxhlet apparatus using 100ml of solvent. Solvents used for extraction were Petroleum ether (60^0C–80^0C), Chloroform (61^0C), Ethanol (78.5^0C) and Aqueous (80^0C) as solvents (Fong, 1973) and the extracts were dried. The dried extracts were stored in a refrigerator at 4^0C.

Phytochemical screening

The extracts were subjected to Preliminary phytochemical Screening methodology were adapted from Kemp (1986) and Sofowara (1982) method. The test for alkaloids was carried out by subjecting 0.5g aqueous extract in 5ml 1% HCl, boiled , filtered and Mayer's reagent was added (Harbone, 1973, Trease and Evans, 1980 and Tewari et al., 1992). The presence of flavanoides was determined using one ml of extract was added with a few drops of neutral ferric chloride solution. The extract was also tested for anthrocyanin by adding a drop of concentrated Sulphuric acid to one of extract. The presence of carboxylic acid was determined by one ml of each extracts was separately treated with a few ml saturated solution of sodium bicarbonate. The presence of coumarins is determined by one ml of each extract was treated with 1ml of alcoholic hydroxide .one ml of various extracts were dissolved in 5ml of alcohol was treated separately with a few drops of neutral ferric chloride solution to find the presence of phenols. The test for steroids, phytosterols was carried out by 1ml each of concentrated Sulphuric acid was added to the extract and allowed to stand for 5minutes.xanthoproteins was detected by taking 1ml of various extracts were treated separately with a few drops of concentrated nitric acid and ammonium solution.

Antimicrobial activity

All the three plants with four extracts were tested against six pathogenic bacterial strains, three Gram positive bacteria (*B.cereus, B. subtilis, S.aureus)* and three Gram negative bacteria (*E. coli, P .vulgaris, S.aureus* and *P.aeruginosa)* by disc diffusion method (Bauer et al., 1986). 20ml of sterilized nutrient agar medium for *E.coli, P.aeruginosa, S.aureus, B.subtilis, B. aureus* and *P. vulgaris* were poured into each sterile petridish. After solidification, the sterile cotton swab was dipped into the broth of these bacteria. The entire agar

surface of each plate was inoculated with this swab, first in the horizontal direction and then in a vertical direction, which ensure the even distribution of organism over the agar surface. The filter paper discs soaked in the plant extract were placed on the surface of the bacteria seeded agar plates and then the plates were incubated at 37^0C for 24h. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around each disc.

Results and Discussion:

The extracts were subjected to preliminary phytochemical screening and the results were tabulated in Table 1.

Table1: Phytochemical screening of some medicinal plants

Phytochemical compounds	***Aegle marmelos***				***Albizzia libbeck***				***Lawsonia inermis***			
	1	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**
Alkaloids	-	+	-	-	+	-	-	+	+	-	-	-
Carboxylic acid	-	+	-	-	-	+	-	-	-	-	-	-
Coumarins	-	-	-	-	+	-	-	-	-	+	_	-
Flavanoids	=	-	-	-	-	-	-	-	-	-	-	-
Anthocyanins	+	-	+	+	+	-	-	+	+	-	+	-
Phenols	+	+	+	+	=	+	+	+	+	+	+	+
Sterols	+	-	-	-	+	-	-	-	+	-	-	-
Xanthoproteins	-	+	+	-	-	-	-	+	-	-	+	-

Solvents: 1. Petroleum ether; 2. Chloroform; 3. Ethanol; 4.Aqueous

\+ _ Positive

_ _ Negative

Chloroform extract of *Aegle marmelos*, petroleum ether, aqueous extract of *Albizzia libbeck* and petroleum ether extract of *Lawsonia inermis* shows the presence of alkaloids. Chloroform extract of *Aegle marmelos* and *Albizzia libbeck* indicate the presence of carboxylic acids. Petroleum ether extract of *Albizzia libbeck* and chloroform, ethanol extract of *Lawsonia inermis* indicates the presence of coumarins. Phenols are present in all the extracts of three plants. Flavanoids is present only in the petroleum ether extract of *Aegle marmelos.* Petroleum ether extract of *Aegle marmelos, Albizzia libbeck* and Lawsonia *inermis*, ethanol extract of *Aegle marmelos*, *Lawsonia inermis* and aqueous extract of *Albizzia libbeck* indicate the presence of anthocyanin. Saponins present in the chloroform extract of *Aegle marmelos*, petroleum ether extract of *Albizzia libbeck* and *Lawsonia inermis* and aqueous extract of *Albizzia libbeck*, *Aegle marmelos* and *Lawsonia inermis*. Sterols are present only in the petroleum ether extract of three plants. Chloroform extract of *Aegle marmelos*, ethanol extract of *Aegle marmelos*, *Lawsonia inermis* and aqueous extract of *Albizzia libbeck* indicate the presence of xanthoproteins

Table 2 shows that the antibacterial activity of petroleum ether extract of Lawsonia *inermis* showed maximum zone of inhibition (3.8cm) against *Bacillus cereus*. The petroleum ether extract of *Albizzia libbeck* showed maximum zone of inhibition (2.8cm) against *B.subtilis*. The maximum antibacterial efficiency was found to be present in petroleum ether extract of *Aegle marmelos* and *Lawsonia inermis* against *P.aeruginosa*. Petroleum ether extract of *Aegle marmelos* has showed no activity against *B. cereus* compare to other plants. Petroleum ether extracts of three plants exhibit the similar inhibition zone (2.6±0.3 and 1.7± 0.2) against *E.coli* and *S.aureus* respectively. Similar result were obtained from the antibacterial activity against *E.coli* indicates inhibitory activity of extract of *Nostoc muscorum* to a greater extent as compared to Streptomycin (Jai Prakash and Hosmani, 2003). Similar result was obtained from the petroleum ether extract of *Combretum albidum* showed antibacterial activity against *B.subtilis*, *S.aureus* and *E.coli* (Karuppusamy and Karmegam, 2001).

Table 2: Antibacterial activity of petroleum ether extract of *Aegle marmelos*, Albizzia *libbeck, Lawsonia inermis* and standard antibiotics (St-Streptomycin, Sp-Spectinomycin)

Microorganisms	Zone of inhibition in Cm					
	Aegle marmelos	***Albizzia libbeck***	***Lawsonia inermis***	**St**	**Sp**	**Control**
B.cereus	-	1.5	3.8	2.7	3.0	-
B.subtilis	2.9	2.8	3.0	2.5	2.9	-
S.aureus	1.7	2.0	1.6	1.6	2.0	-
E.coli	2.9	2.6	2.3	2.8	3.1	-
P.aeruginosa	3.1	1.8	3.1	2.6	3.0	-
P.vulgaris	1.8	1.5	3.3	2.6	3.0	-

Table 3 : Antibacterial activity of chloroform extract of *Aegle marmelos, Albizzia libbeck, Lawsonia inermis* and standard antibiotics (St-Streptomycin, Sp-Spectinomycin)

Microorganisms	Zone of inhibition in Cm					
	Aegle marmelos	***Albizzia libbeck***	***Lawsonia inermis***	**St**	**Sp**	**Control**
B.cereus	-	-	1.1	2.7	3.0	-
B.subtilis	1.4	-	1.0	2.5	2.9	-
S.aureus	-	-	1.0	1.6	2.0	-
E.coli	1.6	-	1.3	2.8	3.1	-
P.aeruginosa	-	-	1.0	2.6	3.0	-
P.vulgaris	-	-	0.8	2.6	3.0	-

Table3 shows that the antibacterial activity of chloroform extract of *Aegle marmelos* showed maximum zone of inhibition (1.6cm) and also the *Lawsonia inermis* showed maximum zone of inhibition (1.3cm) against *E.coli* and *B.subtilis*. The *Albizzia libbeck* showed no inhibitory action against six pathogens. Chloroform extract of *Lawsonia inermis* exhibit activity against all pathogens. Similar result was obtained from chloroform extract of *H.mariflium Retz*. showed antibacterial activity against E.coli and P.aeruginosa (Radha et al., 2003). No activity was found to be against *P.aeruginosa* and *S.aureus* in chloroform extract of *Aegle marmelos*. Similar result was obtained from the antibacterial activity of Aegle *marmelos* **(**Prema, 2004).

Table 4 : Antibacterial activity of ethanol extract of *Aegle marmelos, Albizzia libbeck, Lawsonia inermis* and standard antibiotics (St-Streptomycin, Sp-Spectinomycin)

Microorganisms	Zone of inhibition in Cm					
	Aegle marmelos	***Albizzia libbeck***	***Lawsonia inermis***	**St**	**Sp**	**Control**
B.cereus	1.7		1.3	2.7	3.0	-
B.subtilis	1.6	-	1.2	2.5	2.9	-
S.aureus	1.1	-	1.2	1.6	2.0	-
E.coli	1.4	-	1.3	2.8	3.1	-
P.aeruginosa	2.1	-	0.8	2.6	3.0	-
P.vulgaris	1.5	-	-	2.6	3.0	-

Table 4 shows that the antibacterial activity of ethanol extract of *Lawsonia inermis* showed maximum zone of inhibition (1.3cm) against *E. coli* and *B. cereus. Aegle marmelos* showed maximum zone of inhibition (2.1cm) against *P.aeruginosa*. The *Albizzia libbeck* showed no inhibitory action against six pathogens. Similar result was obtained from the ethanol extract of *Aegle marmelos* showed antibacterial activity against *P.aeruginosa* and *S.aureus* (Prema, 2004).

Table 5 : Antibacterial activity of aqueous extract of *Aegle marmelos*, *Albizzia libbeck*, *Lawsonia inermis* and Standard antibiotics (St-Streptomycin,Sp-Spectinomycin)

Microorganisams	Zone of inhibition in Cm					
	Aegle marmelos	***Albizzia libbeck***	***Lawsonia inermis***	**St**	**Sp**	**Control**
B.cereus	0.7	0.6	2.1	2.7	3.0	-
B.subtilis	0.6	0.6	1.9	2.5	2.9	-
S. aureus	0.6	0.7	1.8	1.6	2.0	-
E.coli	-	0.6	2.1	2.8	3.1	-
P.aeruginosa	0.7	0.6	1.8	2.6	3.0	-
P.vulgaris	0.7	1.1	1.8	2.6	3.0	-

Table 5 shows that the antibacterial activity of aqueous extract of *Lawsonia inermis* showed maximum zone of inhibition (2.1cm) against *E. coli* and *B. cereus*. *Aegle marmelos* showed maximum zone of inhibition (0.7cm) against *P.aeruginosa* and *P. vulgaris*. *Albizzia libbeck* showed maximum zone of inhibition 1.1cm against *P.vulgaris*. A significant effect against all pathogens was observed in the aqueous extracts of Albizzia *libbeck* and *Lawsonia inermis* except *E. coli* in *Aegle marmelos*. Similar result was obtained from the aqueous extract of *Combretum albidum* and *Rhinacanthus nasutus* showed antibacterial activity against *B.subtilis*, *S.aureus* and *E.coli* (Karuppusamy and Karmegam, 2001). Similar result was obtained from the antibacterial activities of Ocimum species **(**Schraffer et al., 1989) and *Lawsonia alba* (Islam et al., 1992).

Among the four solvents used in the study, petroleum ether was considered as the effective one. Because the petroleum ether extract exhibited maximum zone of inhibition against all pathogens compare to the other solvents. Antibacterial activity against six pathogens indicates inhibitory activity of petroleum ether extract of *Aegle marmelos*, *Albizzia libbeck* and *Lawsonia inermis* to a greater extent as compared to Streptomycin. All solvents were also used for the test as Control but showed no inhibited action against pathogens signifying that it serves as a dilutant.

Conclusion:

From the results of antibacterial screening of four solvents (petroleum ether, chloroform, ethanol, aqueous) used in this study, petroleum ether was exhibited best antibacterial activity. Among the three plants used in the study *Lawsonia inermis* was considered as the most effective. Because *Lawsonia inermis* exhibited maximum zone of inhibition against all pathogens compare to other plants, may be due to the presence of alkaloids, anthocyanin, phenols, xanthoproteins, flavanoids, carboxylic acids, coumarins and sterols. The inability of extracts of some selected plants to demonstrate any visible activity against some bacteria may probably be due to the low concentration of the extracts.

In this endeavor, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serves further global needs. The drugs derived from herbs may have the possibility of using in medicine because of its good antibacterial activity. Further research in this pursuit, focusing on the isolation of individual compounds and finally subjecting to clinical trails promises to open new avenues in the use of plants for therapeutic purpose.

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