

Preliminary Phytochemical Screening & Recuperative Effect On Mixing Antibiotics & Antimicrobial Activity Of *Aegle marmelos*

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Abstract : The present investigation is part of continuing programme related to the phytochemical screening of *Aegle marmelos* and effect of antibiotics mixing with the *Aegle marmelos* (herbal extracts) their enhanced potential on microbes. In the world the local plants used in Ancient Indian Medicine, Ayurveda, Siddha and Yunani, in several countries including India several plant species are administered orally to control the various diseases. Some of these plants have been pharmacologically provided to be of some value and may be a popular remedy for the treatment of disease.

From the very beginning of human existence, man has familiarized himself with medicinal plants and used them in a variety of ways throughout the ages. In search of food and to cope successfully with human suffering, primitive man began to distinguish those plants suitable for nutritional purpose from others with definitive pharmacological action. This relationship has grown between plants and man, and many plants came to be used as drugs. The growth of knowledge to cure disease continues at an accelerating pace, and number of new plant-derived drugs increase likewise. Herbal medicine is currently experiencing a revival in Western society, along with other complementary therapies such as traditional Chinese Medicines, Osteopathy and Homeopathy. In this context, the present study is the first milestone with particular emphasis on the application of *Aegle marmelos* the medicinal plant and their effect in mixing with antibiotics for their better formulation and controlling the various diseases in near future.

Keywords: *Aegle marmelos*, *S.aureus*, *S.epidermis*, *E.coli*, *B.subtilis*, Roxythromycin, Cefixime, Levofloxacin, Methanol.

Introduction

The use of plants as medicines represents the biggest human use of the natural world; plants provide the predominant ingredients of medicines in most medical traditions. There are vast number of medicinal plant on earth, the number and percentage for countries and regions vary greatly. Based on positive therapeutic results, herbal medicines are gaining popularity worldwide for human wellbeing and healthcare. One major hurdle that might impair their potential future are "medicine of choice" is the lack of standardization. The breakthrough in chemical marker and identification promise herbal medicines a challenging era. For further classification, the

treatment may be compared using poly herbal syrup, with the various antibiotics mixing and their effectiveness. Thus, it is concluded that the efficacy of mixing up of antibiotics and its bioactive compounds may have more significant effect on the various diseases and antimicrobial activities.

In view of the above considerations the present study was designed to investigate the protective effect and their antibiotics mixing of Moreover, antimicrobial activity content were seen.

Aegle marmelos is naturally distributed in India, Myanmar and Sri Lanka and widely cultivated in Southeast Asia and Tropical Africa, commonly known as Bael, belonging to the family Rutaceae, is a sacred tree for Hindu Religion, is used in different system of medicine, especially Ayurveda, Unani and Homeopathy. In English it is known as Bengal quince, stone apple and golden apple. It is known as *bael* and *bilwa* in Sanskrit and by different names such as *maredu* (Andhra Pradesh) *bael* (Bengal), *bil* (Gujarat), *bael*, *bil* (Himachal Pradesh), *bilpatra*, *kumbala*, *malura* (Karnataka), *kuvalam*, *vilwam* (Kerala), *kuvalum* (Tamilnadu).

Every part of the plant has medicinal properties. The root is an important ingredient of the 'Dasmula' (ten roots) recipe S.S. Agarwal *et al.*, (2005). It is a medium sized deciduous thorny tree with its roots, bark, leaves and fruits of high medicinal value and is cited as one of the red-listed medicinal species of South India, due to its overexploitation in Ayurvedic medicines. The decoction of the root and root bark is useful in intermittent fever hypochondriasis, and palpitation of the heart, I. Lampronti, D *et al.*, (2003).

The leaves and bark have been used in medicated enema. The leaves are also used in diabetes mellitus. The greatest medicinal value, however, has been attributed to its fruit and the unripe fruit is said to be an excellent remedy for diarrhoea and is especially useful in chronic diarrhoea R. Mazumder, S. *et al.*, (2006), G.N. Sharma, *et al.*, (2011). The constituents of *Aegle* are used in heart diseases (Kakiuchi *et al.*, 1991), inflammatory and wound healing (Udupa *et al.*, 1994). Leaves of *A. marmelos* have been reported as hypoglycemic effect (Santhoshkumari and Devi 1990; Sharma *et al.*, 1996). The essential oil from the leaves of *A. marmelos* is known to exhibit antifungal properties (Renu *et al.*, 1986; Rana *et al.*, 1997).

Scope Of Our Study

In this study the use of *Aegle marmelos* for the medicinal purpose used locally in the treatment of various diseases and we examined for their effect on mixing with antibiotics and their antimicrobial activity.

The results of our studies conducted and herewith we report that *Aegle marmelos* is useful in controlling the antimicrobial activity and it would be more helpful when given with mixing up with antibiotics and may be further strengthening the antimicrobial potential. Therefore, the present investigation is part of continuing programme related to the biochemical screening of local plants.

Materials And Methods

Chemicals

All the fine chemicals were purchased from Sigma chemical co., USA. All other chemicals used were of good quality and analytical grade.

Phytochemical Analysis

Qualitative analysis of phytonutrients was done for methanolic extract.

Test for carbohydrates

A small quantity of extract was dissolved separately in 5 ml of distilled water and filtered. The filtrate was tested to detect the presence of carbohydrates.

Molisch's test:

i) To 2ml of extract, 2 ml of Molisch's reagent was added. Then, 2 ml of concentrated sulphuric acid was added along the sides of the test tubes. Disappearance in color on the addition of excess solution indicated the presence of carbohydrates.

Benedict's test:

i) To 0.5 ml of extract, 5 ml of Benedict's reagent was added. The mixture is then boiled for 5 minutes. Presence of a bluish green precipitate indicated the presence of carbohydrates.

Test for Glycosides:

i) To 2ml of extract 1ml of aqueous NaOH solution was added. The appearance of a yellow color indicated the presence of glycosides.

Test for Proteins and Amino acids**Ninhydrin test:**

i) A small quantity extract solution was boiled with 0.2% solution of Ninhydrin. Purple color indicated the presence of free amino acids

Test for Phytosterols and Triterpenoids**Salkowski test:**

i) To 2 ml of the extract, 1 ml of concentrated Sulphuric acid added. Chloroform was added along the sides of the test tube. A red color produced in the chloroform layer indicated the presence of Phytosterols or if it is yellow in color at the lower layer indicated the presence of triterpenoids.

Zinc hydrochloride reduction test:

i) The extract was treated with mixture of zinc dust and concentrated hydrochloric acid. Red color indicated the presence of flavanoids.

Test for Alkaloids

i) A small portion of the solvent free extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with Mayer's reagent (Potassium mercuric iodide solution). The cream precipitate indicates the presence of alkaloids.

ii) Dried powder of herb treated with 5% Ammonical Ethanol and the test carried out after 48 hours, the fraction was treated with Mayer's, Wagner's and Dragndroff's reagent.

Test for Tannins**Gelatin test:**

i) To 5ml of extract, few drops of 1 % lead acetate were added. Absence of a yellow or red precipitate indicated the absence of tannins.

ii) 5gm of extract in 50ml water and boiled for 45 min. in waterbath and 2% gelatin solution is added dropwise.

Test for Saponins:

i) To 5 ml of the extract, a drop of sodium bicarbonate was added. It is shaken vigorously and kept undisturbed for 3 minutes. Appearance of a honey comb like froth indicated the presence of saponins.

Proanthocyanidin:

i) 2ml of extract add 5ml of 2N HCL and kept in water bath for 30 minutes. Then the mixture was cooled and shaken with amyl alcohol.

Iridoids:

i) 1ml of extract add 5ml of aqueous HCl and kept for 3-6 hours, 0.1 ml of extract is decanted and macerate and treated with trim Hill reagent.

Flavonoids:

i) 1ml of extract add 10ml of 95% ethanol and kept in boiling waterbath for 15 minutes and after filtration mg ribbon were added along with 2-3 drops of HCl.

Steroids:

i) 1ml of extract was extracted with methanol for 15minutes and then Libermann Burchard reagent was added drop wise.

Preparation Of Plant Extract

500 mg of the herbal powder samples were separately taken in conical flasks along with 10ml of Methanol. The mixture was then allowed to stand overnight and after that the extract was filtered out. This procedure was repeated thrice. The solids obtained were reconstituted such that the final concentration set was 100mg/ml till complete extraction was ensured.

Preparation of the combinations of the three antibiotics such as Roxythromycin, Cefixime and Levofloxacin were dissolved in double distilled water. Various mixtures were made in combination with the *Aegle marmelos* extract. The *Aegle marmelos* leaves extract was abbreviated as AMLE.

Antimicrobial Activity

The microorganisms were collected from the Microbiology Lab from the Government Hospital - Tirupattur, Tamilnadu. The antimicrobial activity was evaluated using disc and streak plate method.

Test Microbes Used

- i) *Staphylococcus aureus*
- ii) *Staphylococcus epidermis*
- iii) *Escherichia coli*
- iv) *Bacillus subtilis*

Preparation Of The Medium

2.8gms of nutrient agar was weighed correctly and dissolved in 100ml of sterile distilled water. pH was adjusted to 7.2 and was autoclaved at 121°C for 15 minutes. 20ml of molten agar was poured in to the sterile petri plate and allowed to solidify.

Susceptibility Tests

DISC Method

The Whatmann No.1 filter paper discs were made and the plate method is the most commonly used technique for determining susceptibility of micro organisms to know the concentration of antibiotics. Whatmann No.1 paper disc impregnated with combination extraction agents were placed upon the surface of pre inoculated plate. The plates were incubated at 37°C for 24 hours. Susceptibility of effectiveness was observed by the diameter of the inhibition zone around the Disc. Organisms which grew up to the edge of the disc were resistant.

Streak Plate Method

Petri plate containing Nutrient Agar was seeded with the combination mixture and allowed to solidify. Overnight grown cultures were then taken one by one and streaked on the plate. A control plate containing only nutrient agar devoid of antibiotic mixture was simultaneously streaked and incubated. The growth was observed against the control plate.

Sample Preparations

Table 1:

Sample Name	Combinations
A1	AMLE (500mg) + Methanol (10ml)
A2	AMLE + Roxythromycin
A3	AMLE + Cefixime
A4	AMLE + Levofloxacin

Table 2: DISC Method

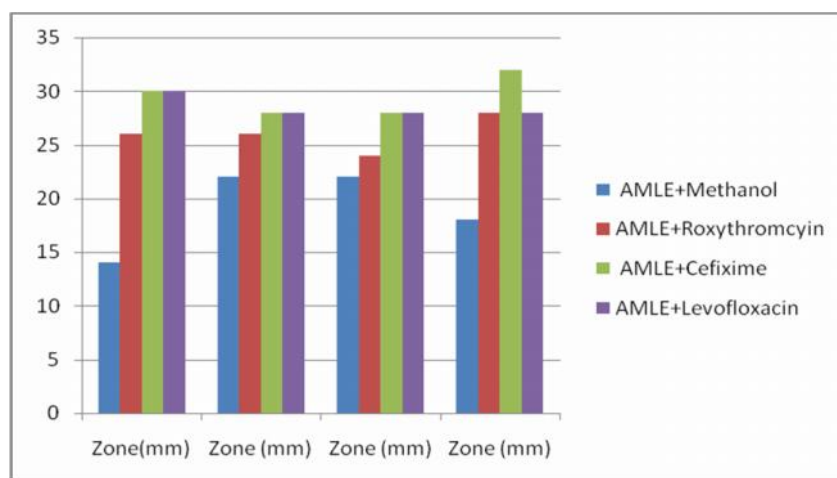
Sample Name	Zone (mm)	Interpretation	Zone (mm)	Interpretation	Zone (mm)	Interpretation	Zone (mm)	Interpretation
AMLE + Methanol	14	I	22	S	22	S	18	S
AMLE +Roxythromycin	26	S	26	S	24	S	28	S
AMLE + Cefixime	30	S	28	S	28	S	32	S
AMLE + Levofloxacin	30	S	28	S	28	S	28	S

Zone: - <8 R – Resistant 8 to 16 I- Intermediate >16 S- Sensitive.

Table 3: Streak Plate Method

Sample Name	Microbes	100mg/ml	250mg/ml	500mg/ml	750mg/ml	1g/ml
AMLE + Methanol	<i>S.aureus</i>	++	+++	++	+++	+++
AMLE + Methanol	<i>S.epidemis</i>	++	++	++	++	+++
AMLE + Methanol	<i>E.coli</i>	+	++	+	+	++
AMLE + Methanol	<i>B.subtilis</i>	+	++	+	++	++
AMLE+ Roxythromycin	<i>S.aureus</i>	+++	+++	+++	+++	+++
AMLE+ Roxythromycin	<i>S.epidemis</i>	+++	+++	+++	+++	+++
AMLE+ Roxythromycin	<i>E.coli</i>	+++	+++	+++	+++	+++
AMLE+ Roxythromycin	<i>B.subtilis</i>	+++	+++	+++	+++	+++
AMLE + Cefixime	<i>S.aureus</i>	+	+	++	+	+++
AMLE + Cefixime	<i>S.epidemis</i>	+	+	+	+	+++
AMLE + Cefixime	<i>E.coli</i>	+	+	+	++	+++
AMLE + Cefixime	<i>B.subtilis</i>	+	+	+++	++	+++
AMLE + Levofloxacin	<i>S.aureus</i>	+++	++	++	++	++
AMLE + Levofloxacin	<i>S.epidemis</i>	+	++	+++	++	++
AMLE + Levofloxacin	<i>E.coli</i>	++	++	++	+++	++
AMLE + Levofloxacin	<i>B.subtilis</i>	++	++	+++	+++	++

+ Less inhibition, ++ Medium, +++ Complete, ---- No inhibition.

Graph 1. Effect of mixing antibiotics and their antimicrobial activity

Discussions

The plants gives slow effect on the microbes but when it is combined with the drugs mixture gives high zone of inhibition. Although the plant extract gives high effect on *Staphylococcus epidermis* and *Escherichia coli* the effect become higher when combine. Table-3 Shows the different concentrations of extract and combination. It helps to find out the perfect concentration of drugs which gives highest inhibition of pathogens.

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Conclusion

There should be a wide range of search for the alternative remedies to prevent and cure the infections and to the antimicrobial resistant microbes. The allopathic forms of antimicrobials give adverse effects to the human system and to the metabolism. So, therefore we need an antimicrobial which has lower side effects and high effectiveness. The combination of high amount of herbal extracts and low amount of drugs will give new path in medicinal world. It is a new concept to combine herbal and Allopathy drugs to be known as herbo-allopathy combinations.

The antimicrobial drug preparations with the herbal extracts will be the new alternative medicines for synthetic drugs. It is suggested that using the extracts are effective and economic, herbal in drugs may be prepared for pathogenic infections. The preliminary phytochemical screening also supported antimicrobial activity. The broad spectrum of antimicrobial activity of *Aegel marmelos* is highly promising for further bioactive compounds can be made evaluated and being continued for the further studies.

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