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Antimicrobial Activity and Phytochemical Analysis of *Aeglemarmelos* (Linn.)In Combination With Commercial Antibiotics.

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Abstract
The present work was carried out to study antimicrobial activity and phytochemical analysis of leaves and fruit extract of *Aegle marmelos* in combination with commercial antibiotic tetracyclin and streptomycin. Ethanol, acetone and aqueous extract of leaves and fruit were tested against *Escherichia coli*, and *Staphylococcus aureus* which are known to be resistant to various antibiotics. These extracts were prepared from fresh *Aegle marmelos* leaves and fruit. These extracts were evaluated for their part in increasing antibacterial activity of tetracyclin and streptomycin against *Escherichia coli*, and *Staphylococcus aureus*. The antibacterial activity of Tetracyclin and Streptomycin was enhanced against the test organism in the presence of these extracts. Tetracyclin and Streptomycin in combination with these extracts showed maximum inhibition against test organism. Phytochemical analysis gave positive results for triterpenoids, alkaloids, Tannins, Saponins, reducing sugar, carbohydrates, Protein and amino acids, phenolic compounds. Leaf extract of *Aegle marmelos* contains pharmacologically bioactive constituents that may be responsible for its activity against test organisms.
Keywords: *Aegle marmelos*, phytochemical analysis, bioactive constituents.

1. Introduction

Plants continue to be a major source of commercially consumed drugs. The continued emergence or persistence of drug resistant organisms and the increasing evolutionary adaptations by pathogenic organisms to commonly used antimicrobials have reduced the efficacy of antimicrobial agents currently in use. In addition to this, antibiotics are associated

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with adverse effects, therefore, the search for new drugs from novel sources, such as plants, is necessary. It has been pointed out that more than 80% of world's population depends on plants to meet their primary health care needs (WHO, 2002). Plants continue to be a major source of commercially consumed drugs. Because of the growing recognition of natural products the demand for medicinal plants has been increasing all over the world. They have minimal toxicity, are cost effective and pharmacologically active, and provide an easy remedy for many human ailments as compared to the synthetic drugs which are a subject of adulteration and side effects (Lee, *et al.*, 2007).

Aegle marmelos (Linn.) belongs to family Rutaceae, commonly known as bael (Hindi) and golden apple (English). It is found throughout India and is known from pre-historic time. *Aegle marmelos* has been used from time immemorial in traditional systems of medicine for relieving constipation, diarrhoea, dysentery, peptic ulcer and respiratory infections (Nadkarni, 2000). Every part of *Aegle marmelos* plant such as its fruits, stem, bark, and leaves possesses medicinal property and is used for treating various eye and skin infections (Kingston, *et al.*, 2009). Leaf is considered to be one of the highest accumulatory parts of the plant containing bioactive compounds which are synthesized as secondary metabolites (Cowan, 1999). More than 100 phytochemical compounds have been isolated from various parts of the plant, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids and tannins. These compounds are well known to possess biological and pharmacological activity against various chronic diseases such as cancer and cardiovascular and gastrointestinal disorders (Badam. *et al.*, 2002). Antioxidant, antiulcer, antidiabetic, anticancer, antihyperlipidaemic, anti-inflammatory, antimicrobial and antispermatogenic effects have also been reported on various animal models by the crude extracts of this plant.The present study was therefore, aimed at evaluating the phytochemical potential and antibacterial activity of *Aegle marmelos* aqueous, ethanolic and acetone extract of leaves and fruit in combination with tetracyclin and streptomycin.

2.Materials and methods

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Sample collection

The fresh leaves and fruit of *Aegle marmelos* were obtained fromJalna city(MS). Plant materials were washed under running tap water to remove surface dirt and impurities, followed by distilled water. These leaves were air dried. After drying the leaves were chopped to get fine pieces. These pieces were used for preparation of different plant extracts. Fruit was crushed in grinder and grinded material was used for preparation of different plant extracts.

Preparation of leaf extracts

Extracts were dissolved in acetone, ethanol and water (1:10); 10 g sample should be dissolved in 100 ml of solvent and kept overnight. After that this was filtered through Whatman's filter paper and filtrate was used as an *Aegle marmelos* extract. These extracts were stored at 4^0C for further use.

Test organism

Bacterial cultures were selected from American type culture collection (ATCC). The strain used for the study were *staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922). These were grown on their respective selective media and purity was determined by morphological and biochemical characterization.

Inoculum preparation

Loopful of pure culture from selective media was picked up and inoculated in Muller Hinton Broth (Himedia). It was incubated at 37 ^{0}C for 3-7 hrs. until moderate turbidity develops. Inoculum turbidity was compared with that of 0.5 McFarland standard.

Preparation of Disc

Whatman's filter paper no.1 was punched to get disc of 6mm diameter. These discs were sterilized under UV light. Each sterile disc was impregnated with ethanol extract, acetone extract, aqueous extract and excess of solvent was dried in controlled temperature.

Antimicrobial activity of extract

The antimicrobial activity of the extract was evaluated by standard disc diffusion method (Baur *et al.,* 2012). Plates of Muller Hinton agar (Himedia) medium having media up to 4 mm were prepared. After solidification lawn of inoculum was prepared on to agar plates for each organism. Inoculum was taken by socking the sterile swab (Himedia) in prepared inoculum of test organisms and spread over the agar plates for respective

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organism. Ethanol extract disc, acetone extract disc and aqueous extract disc of *Aegle marmelos*was applied and incubated at 28-30 ^{0}C for 16-18 hours.

Disc diffusion assay to evaluate combined effects

Disc diffusion method was used to evaluate in vitro antibacterial activity of commercial antibiotics disc (Whatman's paper disc saturated with Tetracyclin, Streptomycin) against test organisms on Muller Hinton Agar (Himedia). To determine combined effect, each paper disc was further impregnated with 10µl of each single extract. Muller Hinton Agar plates were inoculated with test organism. Standard Antibiotics disc were used as positive control and Antibiotics disc impregnated with aqueous, ethanol, and acetone extract were place onto Muller Hinton Agar plate inoculated with test organisms. These plates were incubated 16-18 hours. After incubation, the zones of inhibition were measured.

Assessment of increase in fold area

The increase in fold area was assessed by calculating the mean surface area of the inhibition zone of each antibacterial agent and antibacterial agent plus extract. The fold increase area of different test organism for antibacterial agent and for antibacterial agent plus extract was calculated by equation $(B^2 - A^2)/A^2$, where A and B were zones of inhibition for antibacterial agent and antibacterial agent plus extract, respectively.

Phytochemical analysis

Phytochemical tests were done to find the presence of the active chemical constituents such as alkaloid, flavonoids, glycosides, triterpenoids, steroids, tannin and phenols, reducing sugar, carbohydrates and protein and amino acids by the following procedure. (Kokate; 1986, Harborne,.; 1998, Tiwari.*et.al.;* 2011)

Tests for Alkaloids

To the extract, dilute hydrochloric acid was added, shaken well and filtered. With the filtrate, the following tests were performed.

Mayer's reagent test

To 3 ml of filtrate, few drops of Mayer's reagent were added along sides of tube. Formation of creamy precipitate indicates the presence of alkaloids.

Tests for Carbohydrates

Molisch test

2 ml of extract was treated with 2 drops of alcoholic α-naphthol solution in a test tube and then 1 ml of concentrated sulphuric

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acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

Tests for Reducing Sugars

Benedict's test

Equal volume of Benedict's reagent and extract were mixed in a test tube and heated on a water bath for 5-10 minutes. Solution appears green, yellow or red depending on the amount of reducing sugar present in the test solution which indicates the presence of reducing sugar.

Tests for Flavonoids

Alkaline reagent test

The extract was treated with few drops of sodium hydroxide solution separately in a test tube. Formation of intense yellow color, which becomes colorless on addition of few drops of dilute acid indicates the presence of flavonoids.

Tests for Glycosides

Borntrager's test

To 3 ml of test solution, dilute sulphuric acid was added, boiled for 5 minutes and filtered. To the cold filtrate, equal volume of benzene or chloroform was added and it was shaken well. The organic solvent layer was separated and ammonia was added to it. Formation of pink to red color in ammonical layer indicates the presence of anthraquinone glycosides.

Tests for Tannin and Phenolic compounds

Ferric chloride test

A small amount of extract was dissolved in distilled water. To this solution 2 ml of 5% ferric chloride solution was added. Formation of blue, green or violet color indicates presence of phenolic compounds.

Test for Saponin

Froth test

The extract was diluted with distilled water and shaken in a graduated cylinder for 15 minutes. The formation of layer of foam indicates the presence of saponins.

Tests for Protein and Amino acids

Ninhydrin test

3 ml of the test solution was heated with 3 drops of 5% Ninhydrin solution on a water bath for 10 minutes. Formation of blue color indicates the presence of amino acids.

Tests for Triterpenoids and Steroids

Salkowski's test

The extract was treated with chloroform and filtered. The filtrate was added with few drops of concentrated sulphuric acid, shaken and allowed to stand. If the lower layer turns red, sterol is present. Presence

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of golden yellow layer at the bottom indicates the presence of triterpenes.

3.ResultDiscussion

Table 1.Antibacterial activity of Ethanol, Acetone and Aqueous extract of *Aegle marmelos*

Test organism	Zone of inhibition of *Aegle marmelos*leaf extract in mm			Zone of inhibition of *Aegle marmelos*fruit extract in mm		
	Aqueous	Ethanol	Acetone	Ethanol	Acetone	Aqueous
E.coli	13	20	19	22	20	15
S.aureus	12	18	15	19	15	12

The antibacterial activity of acetone, ethanol and aqueous extract of *Aegle marmelos*against *E.coli* and *S.aureus* were shown in Table 1.Ethanol extract of leaf shows highest 20mm zone against *E.coli* and 18 mm zone against *S. aureus*, followed by acetone extract of leaf shows 19mm against *E. coli* and 15 mm zone against *S. aureus*, whereas aqueous extract of leaf shows 13mm zone diameter against *E. coli* and 12 mm zone against *S. aureus*.Ethanol extract of fruit shows highest 22mm zone against *E.coli* and 19mm zone against *S. aureus*, followed by acetone extract of leaf shows 20mm against *E. coli* and 15 mm zone against *S. aureus*, whereas aqueous extract of leaf shows 15mm zone diameter against *E. coli* and 12 mm zone against *S. aureus*.In the *in vitro* antibacterial activity, streptomycin and tetracyclinan antibacterial agent that is widely used against many bacterial infection was used as positive control for comparison with *Aegle marmelos* extracts. The diameter of zone of inhibition and

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increase in fold area for all the test organism was measured. The antibacterial activity of streptomycin and tetracyclin increased significantly in presence of ethanol, acetone and aqueous extract of *Aegle marmelos* shown in table no.2 to 5.

Table 2. Zone of inhibition of streptomycin against test organism in absence and in presence of *Aegle marmelos* leaf extract at content 20µl per disc

Test Organism	***Aegle marmelos*Extract**	**Streptomycin+Extract (B)**	**Streptomycin (A)**	**Increase in Fold B^2-A^2/A^2 Area**
E.coli	Ethanol	24mm	20mm	0.44
	Acetone	23mm	20mm	0.32
	Aqueous	21mm	20mm	0.1
S.aureus	Ethanol	26mm	23mm	0.27
	Acetone	26mm	23mm	0.27
	Aqueous	24mm	23mm	0.08

Table 3. Zone of inhibition of Tetracyclin against test organism in absence and in presence of *Aegle marmelos* leaf extract at content 20µl per disc

Test Organism	***Aegle marmelos*Extract**	**Tetracyclin+Extract (B)**	**Tetracyclin(A)**	**Increase in Fold B^2-A^2/A^2 Area**
E.coli	Ethanol	26 mm	25mm	0.1
	Acetone	26mm	25mm	0.1
	Aqueous	25mm	25mm	0.0

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S.aureus	Ethanol	26mm	23mm	0.27
	Acetone	28mm	23mm	0.48
	Aqueous	23mm	23mm	0.0

Table 4. Zone of inhibition of streptomycin against test organism in absence and in presence of *Aegle marmelos* fruit extract at content 20µl per disc

Test Organism	***Aegle marmelos*Extract**	**Streptomycin Extract (B)**	**Streptomycin (A)**	**Increase in Fold B^2-A^2/A^2 Area**
E.coli	Ethanol	26mm	20mm	0.69
	Acetone	23mm	20mm	0.32
	Aqueous	22mm	20mm	0.21
S.aureus	Ethanol	26mm	23mm	0.27
	Acetone	26mm	23mm	0.27
	Aqueous	24mm	23mm	0.08

Table 5.Zone of inhibition of streptomycin against test organism in absence and in presence of *Aegle marmelos* fruit extract at content 20µl per disc

Test Organism	***Aegle marmelos*Extract**	**Tetracyclin+Extract (B)**	**Tetracyclin(A)**	**Increase in Fold B^2-A^2/A^2 Area**
E.coli	Ethanol	29mm	25mm	0.34
	Acetone	26mm	25mm	0.1

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	Aqueous	25mm	25mm	0.0
S.aureus	Ethanol	29mm	23mm	0.59
	Acetone	26mm	23mm	0.27
	Aqueous	23mm	23mm	0.0

Table 6.Phytochemical analysis

Phytochemical Compounds	**Leaf**			**Fruit**		
	Ethanol	**Acetone**	**Aqueous**	**Ethanol**	**Acetone**	**Aqueous**
Test for Alkaloids	+	+	+	+	+	-
Test for Carbohydrates	-	-	-	+	-	+
Test for reducing sugars	+	+	+	+	+	+
Test for Flavanoids	-	-	+	-	-	-
Test for Glycosides	-	-	-	-	-	-
Test for Tannins and Phenolic compounds	-	+	+	-	+	+
Test for Saponins	-	+	-	-	+	-

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Test for Protein and Amino acids	+	+	-	-	+	-

Phytochemical analysis

The phytochemical analysis of *Aegle marmelos* extracts using acetone, ethanol and aqueous was showed in Table 6.Phytochemical analysis of *Aegle marmelos* in the solvents such as acetone, athanol and aqueous showed presence of bioactive compounds. Theleaf ethanol extract of *Aegle marmelos* showed the presence of alkaloid, reducing sugar and proteins and amino acids. Presence of triterpenoids and steroids. Alkaloid, saponins, tannin and phenolic compound, reducing sugar were observed in Acetone extract of *Aegle marmelos.* Aqueous extract of *Aegle marmelos* shows presence of flavonoids, Alkaloid, tannin and phenolic compound, reducing sugarThe Fruit ethanol extract of *Aegle marmelos* showed the presence of alkaloid, reducing sugar and carbohydrates. Presence of proteins and amino acids, Alkaloid, saponins, tannin and phenolic compound, reducing sugar were observed in acetone extract of *Aegle marmelos.* Aqueous extract of *Aegle marmelos* shows presence of carbohydrates, tannin and phenolic compound, reducing sugar. Present investigation revealed that fromulation of herbal and synthetic medicine can be used againsthuman pathogenic microorganism. Vlietinck, *et al.*, (1995) reported that the Antibacterial properties of various plant parts like leaves, seeds, and fruits have been well documented for some of the medicinal plants for the past two decades.A variety of compounds is accumulated in plant parts accounting for their constitutive antimicrobial activities.Prasannabalaji *et al.*, (2012) investigated that the newer antibacterial bioactive compounds targetted on the unexplored folk medicinal plants, being used for centuries in treating local population. The plant extracts are considered as best source of bioactive compounds particularly for traditional

healers as they contain components of therapeutic values. The bioactive compounds have been detected for either bacteriostatic or bactericidal property and have very minimum or no toxicity to host.Plants are known to have beneficial therapeutic effects in traditional Indian system of medicine. Much work has been done on ethno medicinal plants in India. It has been suggested that phytochemical extracts from plants are used in allopathic medicine as they are potential sources of antiviral, antitumor and antimicrobial agents (Nair R,*et al.*, 2005; Ramya*et al.*, 2008).In this present study the antibacterial activity was found to be best in fruits and leaves of the *Aegle marmelos* against bacterial pathogens.The antibacterial compound mainly found in *Aegle marmelos* were tannins, proteins and amino acids, alkaloids, flavanoids, saponinsandterpenoids.

4. Conclusion

This research work states that the presence of alkaloids, terpenoids, saponins, tannin, flavonoids, and steroids in the extract of *Aegle marmelos* were responsible for its antimicrobial activity. These compounds exhibit a maximum zone of inhibition against *Escherichia coli*, and *Staphylococcus aureus.* in combination with tetracyclin and streptomycin. Moreover, this study shows that some plants show much promise in the development of phytomedicines having antimicrobial properties. In this endeavor, traditional herbal medicines must perforce be granted the benefits of modern science and technology to serve further global needs. The drugs derived from herbs may have the possibility of use in medicine because of their antibacterial activity. Thus extract alone or their formulation can be used as effective agents against human pathogens.

5. References

1. Badam, L., Bedekar, S. S., Sonavane, K. B., & Joshi, S. P. (2002). In Vitro Antiviral Activity of Bael (Aegle marmelos Corr) upon. *J. commun. Dis*, *34*(2), 88.
2. Baur, R. W., Kirby, M. D. K., Sherris, J. C., & Turck, M. (2012). Antibiotic susceptibility testing by standard single disc diffusion method. *Am J ClinPathol*, *45*, 493-496.
3. Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical microbiology reviews*, *12*(4), 564-582.

4. Harborne, A. J. (1998). *Phytochemical methods a guide to modern techniques of plant analysis*. Springer science & business media.
5. Kingston, C., Jeeva, S., Jeeva, G. M., Kiruba, S., Mishra, B. P., & Kannan, D. (2009). Indigenous knowledge of using medicinal plants in treating skin diseases in Kanyakumari district, Southern India.
6. Kokate, C. K. (1986). Practical pharmacognosy. *Vallabh Prakashan, New Delhi*, *111*.
7. Lee, S. B., Cha, K. H., Kim, S. N., Altantsetseg, S., Shatar, S., Sarangerel, O., & Nho, C. W. (2007). The antimicrobial activity of essential oil from Dracocephalum foetidum against pathogenic microorganisms. *The Journal of Microbiology*, *45*(1), 53-57.
8. Nadkarni, K. M. (2000). Indian MateriaMedica: Popular Prakashan Private Limited Publication. *Mumbai*, *3*, 619-622.
9. Nair, R., KALARIYA, T., &Chanda, S. (2005). Antibacterial activity of some selected Indian medicinal flora. *Turkish Journal of Biology*, *29*(1), 41-47.
10. Prasannabalaji, N., Muralitharan, G., Sivanandan, R. N., Kumaran, S., &Pugazhvendan, S. R. (2012). Antibacterial activities of some Indian traditional plant extracts. *Asian Pacific Journal of Tropical Disease*, *2*, S291-S295.
11. Ramya, S. (2008). In Vitro Evaluation of Antibacterial Activity Using Crude Extracts of Catharanthusroseus L.(G.) Don. *Ethnobotanical Leaflets*, *2008*(1), 140.
12. Tiwari, P., Kumar, B., Kaur, M., Kaur, G., & Kaur, H. (2011). Phytochemical screening and extraction: a review. *Internationale pharmaceuticasciencia*, *1*(1), 98-106.
13. Vlietinck, A. J., Van Hoof, L., Totte, J., Lasure, A., Berghe, D. V., Rwangabo, P. C., &Mvukiyumwami, J. (1995). Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *Journal of ethnopharmacology*, *46*(1), 31-47.
14. World Health Organization. (2002). WHO traditional medicine strategy 2002-2005.