Study of Antibacterial property in variant of vilvam oil (*Aegle marmelos)* against pathogenic Bacteria.

Swati C.Guleria & Shubhika Jain & Pulak Das*
Adithya Biotech lab & Research Pvt Ltd, Chandandih, Nandanvan Road, Raipur, 492001, Chhattisgarh, India.

Received: March 11, 2019 Accepted: April 21, 2019

ABSTRACT: : *In developing countries including India, infectious diseases accounts for high proportion of health problems. As various microorganisms have developed resistance to many antibiotics due to indiscriminate use of commercial antimicrobial drugs used for the treatment of infectious disease, Because of that researchers are progressively turning their attention to develop better drugs against microbial infections and screening of medicinal plants for their antimicrobial properties. Medicinal plants serve as a potential source of bioactive compounds which have been acknowledged by the pharmaceutical studies. Vilvam is a medium-sized, armed, deciduous tree from the family Rutaceae. These trees at all stages of maturity are used as medicines against various human ailments. The present study was carried out to determine the antibacterial activity of variant of vilvam oil (AB1and AB2) against selected pathogenic bacteria (B. subtilis ,E. coli, S. aureus). The highest antibacterial activity was shown by AB1 oil against E. coli (29mm) than B. subtilis (28mm), S. aureus. (14mm), and between the two variant oil (AB1 and AB2) AB1 has better antimicrobial property. Thus, antibacterial activity against both type of bacteria, indicate this variant oil (AB1) can be used for the development of various antibiotic drug medicine against antibiotic resistant microorganism.*

Key Words: *Vilvam, Bael, Aegle marmelos (L.), AB1 oil AB2 oil, Antibacterial Property, agar well diffusion.*

INTRODUCTION:

Vilvam is a holly tree commonly found in the Sacred Groves from Kanyakumari to Kashmir in the foot hills. Vilva trees are the Sthalavirusham of the most of the Shiva temples in India. The Botanical Name of the Vilvam is *Aegle marmelos*(L.), which belongs to the Orange family Rutaceae, it is commonly called as Bael in Hindi, Vilvam in Tamil and Bilva in Sanskrit and Bengal quince **(John and Stevenson, 1979)**. This tree was originated in India and is presently growing in most of the countries of Southeast Asia, it also grows wild, especially in dry forest, outer Himalayas, Shivaliks, South Indian Plateau with altitudes ranging from 250-1200m, also found growing along foothills of Himalayas, Uttranchal, Jharkhand, Madhya Pradesh and the Deccan Plateau and along the east coast **(Sharma *et al.,* 2007).** Even though all the parts of the plants are useful, the leaves and fruits are mostly used as important drug in the ancient system of medicine to cure almost all the common ailments of the Human being.The leaves are used as astringent, laxative, febrifuge and expectorant. The leaves are useful in opthalamia, inflammations, catarrh, and asthmatic complaints**(Chakraborty Manodeep 2012**). Extensive chemical investigations on various parts of the tree have been carried out also. Vilvam plant acts as a 'Sink' for chemical pollutants as it absorbs poisonous gases from atmosphere and make them inert or neutral. It is a member of plant species group which is also known as 'climate purifiers', which emit larger percentage of oxygen in sunlight as compared to other plants.

Antimicrobial resistance happens when microorganisms (such as bacteria, fungi, viruses, and parasites) are exposed to antimicrobial drugs (such as antibiotic, antifungal, antiviral, antimalarial, and anthelmintic) and resistant can be exchange between certain types of bacteria. The misuse of antimicrobial medicines accelerates this phenomenon, all classes of microbes can develop resistance. The WHO defines antimicrobial resistance as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection by that microorganism(*Antimicrobial resistance Fact sheet N°194*). It is possible for AMR to develop in bacteria, but it can also originate in fungi, parasites, and viruses. This resistance could affect people with *Candida*, malaria, HIV, and a wide range of other conditions .People who use these drugs are at risk of allowing AMR to develop. This could make them more likely to have a health problem in the future that will not respond to antibiotics. As a result, the medicines become ineffective and infections persist in the body, increasing the risk of spread to others. Without effective antimicrobials for prevention and treatment of infections, medical procedures such as organ transplantation, cancer chemotherapy, diabetes management and major surgery (for example, caesarean sections or hip replacements) become very high risk. Some

bacteria are now so resistant that there are no antibiotics doctors can use to treat the infections they cause. ***Escherichia coli,*** bacterium is a common cause of food-borne disease and urinary tract infections. The rate of antibiotic resistance in *E. coli* is increasing quickly. As infections stop responding to current drugs, there is an urgent need to find alternatives. In some cases, this means using combinations of different medications, known as multiple-drug therapy. There are very few new antibiotics available to replace them. Put simply, the more antibiotics we use, the faster and more serious resistance develops in bacteria. More and more, germs are sharing their resistance with one another, making it harder for us to keep up. According to the Centers for Disease Control and Prevention (CDC), at least 2 million people become infected with antimicrobial-resistant bacteria in the Antimicrobial resistance (AMR), or drug resistance, develops when microbes, including bacteria, fungi, parasites, and viruses, no longer respond to a drug that previously treated them effectively. This would mean that common infections and minor injuries that became straightforward to treat in the 20th century could again become deadly. Because the increase in the occurrence of multiple drug resistance has demanded the search for innovative antimicrobials from natural plant sources. So the new research is focusing on screening of natural products which are mainly found in medicinal plants to develop new and most effective drugs against various infections and microbial diseases. Vilvam has enormous traditional uses against various diseases and many bioactive compounds have been isolated from this plant also (**Maity *et al*., 2009**).Its seeds are beneficial in treating diabetes, high blood pressure and high cholesterol levels. And seed oil also exhibits antibacterial activity against different strains of *Vibrios* (**Kulkarni *et al.,* 2012).** Vilvam fruit is globuse with grey or yellowish hard woody shell. Inside this, there is soft yellow or orange colored mucilaginous pulp,It has numerous seeds, which are densely covered with fibrous hairs and are embedded in a thick, gluey, aromatic pulp (**Kaushik *et al.,* 2002**). In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in various traditional systems. As few studies of plant products have included further investigations of the biological action of antimicrobial plant extracts (**Ahmad *et al*., 2001).** And various bioactive compounds have also been isolated out of it (**Badam *et al.,* 2002, Gupta and Tondon 2004**).The most important ingredients present in plants are alkaloids, terpenoids, steriods, phenols glycosides and tannins (**Venkatesan *et al* 2009**).

The aim of the present study is to compare the antibacterial property variant of vilvam oil (AB1and AB2) against pathogenic bacteria (*Escherichia coli, Bacillus subtillis, Staphylococcus aureus*).

MATERIALS AND METHODS-

Collection of plant materials:

Healthy, fresh, disease-free and mature leaves of *Aegle marmelos* (L.) is collected from **Adithya biotech lab and research pvt. ltd**. Raipur, Chhattisgarh. The leaves were thoroughly washed under running water to remove dust and used for oil extraction process.

Extraction of oil from leaves:

The leaf samples of *Aegle marmelos* (L.)(vilvam) collected were first washed with tap water followed by distilled water and then dried under shade. As 750ml of double distilled water was used with 250 gm of dried plant material for extraction process through Clevenger apparatus. The oil and hydrosol obtained were collected in different bottle's and were stored at 4^0C for further use.

Test microorganism and inoculums preparation:

The microbial strain used for antibacterial assessment was *Bacillus subtilis*(ATCC1813) ,*Escherichia coli*(ATCC11229), *Staphylococcus aureus*(ATCC2112),.The bacterial strain was maintained on nutrient agar, subcultured regularly and stored at 4^0C for further use. One loop full of overnight bacterial culture was inoculated in nutrient broth at 37^0C in incubator for 16-18 hrs.

Antibacterial activity:

The antibacterial activity testing of the selected cultures was carried out according to the method described by (**Perez.*et al* 1990)** with slight modifications. The antibacterial activities of AB oil were determined by using agar-well diffusion method. 200 µl of the standardized cell suspension were spread on Mueller Hinton and Luria agar plates using a sterile spreader. Wells were bored into the agar plates using a sterile cork borer. 100 µl of both the oil was introduced into the well with positive control (Ampicillin 100mg) in another well and the plates were incubated at 37^0C for 24 hrs. The diameters of the zone of inhibition were noted after 24 hrs.

RESULT AND DISCUSSION:

In the present study, comparison is done between the two variant of vilvam oil(AB1and AB2) which was

extracted from the leaves of *Aegle marmelos* (L.)(vilvam) were investigated for the antibacterial activity.

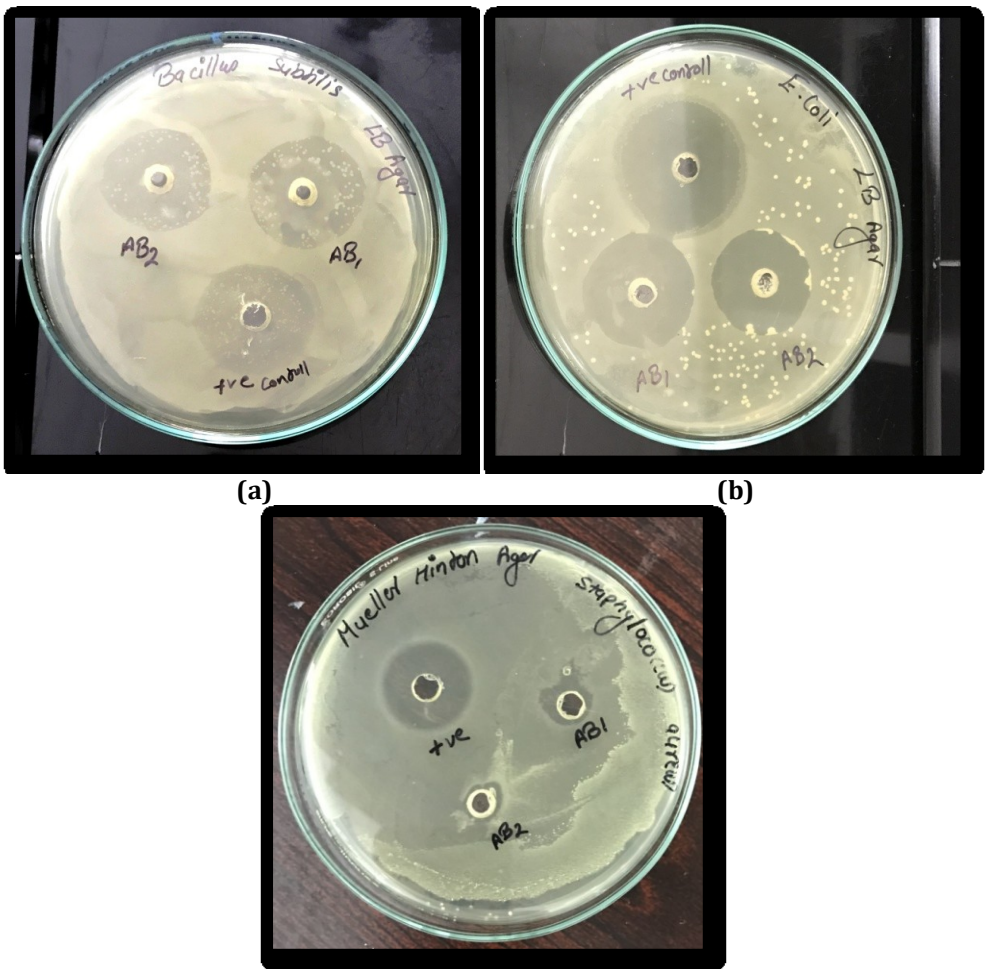
Antibacterial activity:

In the present investigation, it was found that in comparison of AB1and AB2 oil, AB1 oil has more potent antibacterial activity than AB2 oil which is illustrated in **Table (2).** All the evaluated microorganism's showed variable degree of inhibition zones against selected oil's. Based on the zone of inhibition, *Escherichia coil* reported the maximum zone of inhibition (29 mm) **[Fig.2.a]** showed highest antibacterial activity. *Bacillus subtilus* showed (28mm) **[Fig.2.b]** and *Staphylococcus aureus* shows (14mm) **[Fig.2.c]** zone of inhibition. The standard antibiotic, Ampicillin was also found to be active against the test bacteria and taken as positive control.

Table 2: Antibacterial activity of AB1 oil and AB2 oil in *Escherichia coli, Bacillus subtilus ,Staphylococcus aureus.*

Microorganism Used		*Escherichia coli*		*Bacillus subtilus*		*Staphylococcus aureus*	
Conc. (µg/ml)	ZOI (mm)	Quantity (µl)	ZOI (mm)	Quantity (µl)	ZOI (mm)	Quantity (µl)	ZOI (mm)
PC	mm	100µl (100mg)	32mm	100µl (100mg)	29mm	100µl (100mg)	22mm
AB1	mm	100µl	29mm	100µl	28mm	100µl	14mm
AB2	mm	100µl	27mm	100µl	24mm	100µl	10mm

PC- Positive Control (Ampicillin), AB1,AB2- Test Sample, ZOI- Zone of Inhibition, Conc -Concentration.



(a) (b)

(C)

Fig.(2): Antibacterial activity of AB1 oil and AB2 oil against (a) *Escherichia.coli* (b) *Bacillus subtilis* (c) *Staphylococcus aureus*

CONCLUSION:

Our results demonstrated that between variant of vilvam leaf oil (AB1 and AB2) AB1 oil shows great result and intense antimicrobial potential against gram positive and negative bacteria. The findings suggest that the AB1 oil could be used as alternative for development of more effective and efficient antimicrobial drugs from natural source which can be used for treating infectious diseases.

Acknowledgment :

The authors are thankful to the Mr. Swaroop chand Jain,Chairman and Mr.Shailendra Jain, MD of Adithya Biotech Lab & Research Pvt Ltd, Raipur and also R&D team member of the company.

REFERENCES:

1. JohnL, Stevenson V (1979). The complete book of fruit. Angus and Robertson Publishers Sydney.
2. Sharma PC, Bhatia V, Bansal N , Sharma A (2007). A review on Bael Tree. Natural Product Radiance 6(2): 171-178.
3. ChakrabortyManodeep,Patel Axay R, Garach Dipak, Kamath Jagdish V (2012)Aegle marmelos (LINN.): a therapeutic boon for human health. J. Res. Ayurveda and Pharmacy 3:159-163.
4. "Antimicrobial resistance Fact sheet N°194". *who.int. April 2014.* Archived *from the original on 10 March 2015*. Retrieved 7 March 2015
5. Maity P, Hansda D, Bandyopadhyay and Mishra DK (2009). Biological Activity of crude extracts and chemical constituents of bael, Aegle marmelos (L.), Ind J Experimental Biology 47: 849-861.
6. Kulkarni A.S, Khotpal R.R, Karadbhajane V.Y, More, V.I, (2012). Physico-chemical Composition and lipid classes of Aegle marmelos (Bael) and Citrullus colocynthis (Tumba) seed oils. J. Chem. Pharm. Res 4(3): 1486 1488.
7. Kaushik RA, Yamdagni R,Sharma JR, Kenghe RN. (2008). Changes in quality parameters during processing and storage of Bael fruit processing for value addition and employment generation. Food Pack Com 2(8):10-12.
8. Ahmad I, Beg A.Z. (2001). Antimicrobial and Phytochemical Studies on 45 Indian Medicinal Plants against Multi-drug Resistant Human Pathogens. J. Ethnopharmacol. 74: 113-123.
9. Badam L, Bedekar SS, Sonawane KB and Joshi SP (2002). In vitro antiviral activity of Bael (Agle marmelos) upon human coxsackie viruses B1-B6. Journal of communicable 34(2):88-99.
10. Gupta A.K and Tondon N (2004). Review on Indian medicinal plant, Indian council of medicinal research, New Delhi, 312.
11. Venkatesan D, Karrunakarn CM, Selva SK and Palani PTS (2009). Identification of Phytochemical Constituents of Aegle marmelos Responsible for Antimicrobial Activity against Selected Pathogenic Organisms. Ethno botanical Leaflets 13:1362-72.
12. Perez C, Pauli M, Bazevque P.(1990)An antibiotic assay by the agar well diffusion method. Acta Biologiae et Medicine Experimentalis 15:113-115.