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Research Article

A Comparative Study of Anti-Microbial Potential of Ethanolic Extracts Obtained from Leaves of Betel, Beal, Peppermint, And Custard Apple

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ABSTRACT

Antibiotics are vital in treating infections that were once widely fatal and safeguarding procedures that were once unthinkable. But in recent times development of Antibiotic resistance is a global public health problem. Medicinal plants are first known primary source of curative drugs to man since ancient times. In the present study, we have selected four medicinal plants were used to find out the antibacterial potential against aerobic bacteria, namely Annona squamosa, Aegle marmelos, Piper betel, and Mentha arvensis leaves by extracting phytochemicals present in leaves of four plants using ethanol and performed phytochemical screening on the obtained ethanolic extracts which showed presence of alkaloids, glycosides, flavonoids, saponins and terpenes then all the four extracts were evaluated for Anti- Microbial activity using the cup plate method against Escherichia coli bacteria and zone of inhibition were calculated. The inhibition zone for each selected plant varied in diameter from 33mm to 75mm. These medicinal plant extracts produced clear and circular inhibition zones after 24 hours of incubation.

INTRODUCTION

Medicinal plants are rich in a wide range of secondary metabolites with antimicrobial properties, such as saponins, tannins, alkaloids, phenols, glycosides, alkaloids, flavonoids, sesquiterpenes, and terpenoids. ^{[1][2]} Since time immemorial, plants have been resisting the

continuous attacks of microorganisms like Parasites, fungi, Bacteria, and Viruses by producing endless secondary metabolites. With the help of new and emerging technologies, Humans developed a wide range of antibiotics to fight diseases, and disease-causing microorganisms developed new ways to strengthen themselves and live longer, which is termed as Antibiotic

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resistance. However, plants can develop new, antimicrobial agents faster than man-made remedies^[3], and that is why plants have succeeded in their fight against microbes since millions of years while humans have failed. Historically, the ancient civilizations considered plants as the main source of new leads for antimicrobial remedies and pharmaceutical development^[4]. In this present study we have selected four locally available medicinal plants *Annona squamosa*, *Aegle marmelos*, *Piper betle*, *Mentha arvensis*. *Annona squamosa* is one of the most important Indian medicinal plants with edible fruits and is commonly known as the custard apple. This plant belongs to the Annonaceae family and has been used as a traditional medicine for many years, with benefits for patients with various diseases. *A. squamosa* leaves contain phytochemical compounds including carbohydrates, cardiac glycosides, coumarins, tannins, flavonoids, saponins, and nutritional analysis shows that the composition of *A. squamosa* leaves features of proteins, lipids, calcium, and water³. The leaves have demonstrated a wide range of pharmacological properties, including antimicrobial, antifungal, anti-inflammatory, anticancer, antiulcer, antidiabetic, antidiarrheal, antiplatelet, antioxidant, hepatoprotective, neuroprotective, and cytoprotective. The fruits are generally eaten fresh, or used to make juice beverages or sorbet, and are a good source of iron, calcium, and phosphorus.^[5]



Fig no 1 Leaves of *Annona squamosa*

Aegle marmelos has been known to be one of the most important Indian traditional and medicinal plants. The phytochemical compounds are, namely phenols, flavonoids, alkaloids, cardiac glycosides, saponins, terpenoids, steroids, and tannins, and leaves are synthesised as secondary metabolites. The phytochemical compounds contain pharmacological activity against various chronic diseases such as cancer and cardiovascular, Antioxidant, antiulcer, antidiabetic, anticancer, anti-inflammatory, antimicrobial, and gastrointestinal disorders^{[6],[7]}



Fig no 2: Leaves of *Aegle marmelos*

Piper betle leaves are widely used in Indian traditional medicine particularly in ayurveda, and also has culinary and cultural uses. One of the most important constituents contains phytochemical compounds like flavonoids, tannins, phenols, glycosides, reducing sugars, alkaloids and saponins were found in the water extract of betel leaves^[8]. Betel leaves are known for their pungent, aromatic, and spicy flavor. Betel leaves are recognized for various curative properties, including the ability to freshen breath, combat body odor, and address respiratory issues like coughs, throat, and lung problems, to prevent itching caused by fungus and internal/ external bacteria^[9]. The leaf extracts exhibit several biological activities including oral hygiene, anti-diabetic, cardiovascular, anti-inflammatory, anti-ulcer, anti-cancer, hepato-protective, and anti-

infective etc antibacterial, antiseptic, and antifungal agents ^{[10],[11]} anti-cancer^[12] and immunomodulatory^[13] associated with leaf extracts and purified compounds. The betel leaves have promote healing of wounds, aid digestion and positively ^[14]



Fig no 3: Leaves of *Piper betle*

Mentha arvensis is a species of mint, primary source for the production of peppermint. Both peppermint tea and the essential oil derived from *Mentha arvensis* are widely used for their flavor and potential health benefits. Mints are used as flavoring agents in food, beverage, chewing gums, and candies^[15]. It is considered as healthy leafy vegetable because of its vitamins and mineral nutrients^[16]. Due to the presence of potential secondary metabolites, mint was employed to treat various disorders since antiquity in ayurvedic and Chinese medicines¹⁷ the plant leaves contain essential oil of peppermint is used in reduction of swelling, pain reliver, and is often used for the treatment of headache and eye redness, arthralgia, rubella, measles, chest, and hypochasm distension^{[17],[18]} and also repels some pest insects, and mosquitoes. The phenolic compounds of the leaves include rosmarinic acid and several flavonoids, primarily eriocitrin, luteolin, and hesperidin. It is greatly responsible for the spasmolytic nature of peppermint ^[19]. Menthol can be described as promoting or enhancing bile flow, rather than just stimulating it ,

decreasing"lowering," "relaxing," or "attenuating" the tone^[20], act of belching ^[21], exhibiting antimicrobial action,^[22]. polyphenols and the aforementioned essential oils work synergistically to give peppermint its strong antioxidant . The pharmacological activity of peppermint includes antimicrobial and antiviral activities, strong antioxidant and antitumor actions, and some antiallergenic potential.



Figure no 4: Leaves of *Mentha arvensis*

MATERIALS AND METHODS:

Collection of Plant Material: Fresh leaves of *Annona squamosa* (custard apple), *Aegle marmelos* (beal), *Piper betle*, *Mentha arvensis* (peppermint) were sourced from the surroundings of Korangi and dried under shade for fifteen days, and then powdered finely.

Preparation of extracts: 20 g of each plant leaf powder was macerated using 100 ml of ethanol (1:100) for three days with occasional stirring, followed by filtration and steam distillation, which produced ethanolic extracts.

Table No 1 Extraction Yields

Name of the extract	Weight of the extract (gm)
<i>Annona squamosa</i> (ASE)	3.5
<i>Aegle marmelos</i> (AME)	4.0
<i>Piper betle</i> (PBE)	3.0
<i>Mentha arvensis</i> (MAE)	3.5

Phytochemical screening:

The obtained extracts ASE, AME, PBE and MAE were dissolved in 10 ml of distilled water and then tested for phytochemicals present in them by using the following tests

Alkaloids:

Dragendroff's Test: Take 1 ml of test sample in a test tube and add 2ml of Dragendroffs reagent, formation of orange red precipitate indicates the presence of alkaloids.

Mayer's test: Take 1 ml of test sample in a test tube and add 2 ml of Mayers's reagent, formation of cream-colored precipitate indicates the presence of alkaloids.

Wagner's Test: Take 1 ml of test sample in a test tube and add 2 ml of wagner's reagent, formation of reddish brown precipitate indicates the presence of alkaloids.

Glycosides:

Kellar Kilani Test: Take 1 ml of test sample in a test tube and add 2ml of glacial acetic acid, then add few drops of 5% ferric chloride solution, slowly add sulfuric acid, formation of a brown ring at the interface indicates presence of glycosides.

Flavonoids: Take 1 ml of test sample in a test tube add magnesium ribbon and add few drops of concentrated hydrochloric acid, formation of pink color indicates presence of flavonoids.

Shinoda Test: Take 1 ml of test sample in a test tube add magnesium ribbon and add few drops of concentrated hydrochloric acid, formation of pink color indicates presence of flavonoids.

Steroids:

Liebermann buchard test: Take 1 ml of test sample add 1ml of chloroform to this add 1ml acetic anhydride and 1ml of sulphuric acid formation of bluish green color indicates presence of steroids.

Terpenes:

Salkowaski test: Take 1 ml of test sample add 1 ml of chloroform and add 1ml of sulphuric acid, at the interface a brown ring formation indicates presence of terpenes.

Anti-bacterial activity:

Test organism:

Gram-positive organisms used in the study were *Staphylococcus aureus*, and Gram-negative organism used was *Escherichia coli*

Standard used:

Tetracycline was used as a standard for Gram-positive and Gram-negative organisms.

Preparation of test sample solution:

From the extracts ASE, ASE, AME, PBE, and MAE, 1g of each extract was taken and dissolved in distilled water of 100 ml, from which serial dilutions of concentration 25,50,75,100 µg/ml were prepared.

Preparation of Nutrient agar medium:

Definite volumes of peptone, beef extract, and agar were dissolved in distilled water and the pH was adjusted to 7.2. This solution was sterilized by autoclaving at 15 psi for 20 minutes.

Table No. 2: Composition of Nutrient Agar Medium

S.NO	Ingredients	Quantity/100ml
1	Peptone	1.5gm
2	Beef extract	1.5gm
3	Agar	2 gm



4	Sodium chloride	0.5gm
5	Water	100 ml

Sterilization:

Sterilization of the medium, tubes, borer, etc., was done by autoclaving at 15 lbs/inch² for 20 minutes. The glass ware like syringes, Petri dishes, pipettes, and empty test tubes, were sterilized by dry heat in an oven at a temperature of 160°C for 1 hour.

Experimental procedure:

The sterile borer was used to prepare six cups of 8 mm diameter, in the medium of each Petri dish. At the center, one more cup was made for the standard drug tetracycline; its zone of inhibition was

measured to compare with the zone of inhibition of the test drug. All the plates were kept at room temperature for effective diffusion of the test drug and standard. Later, they were incubated at 37 ± 1°C for 24 hours. The presence of definite zones around the cup of any size indicated antibacterial activity. The diameter of the zone of inhibition was measured and recorded.

RESULT AND DISCUSSION:

Phytochemical screening:

All four extracts ASE, AME, PBE, MAE have showed presence of Alkaloids, Glycosides, Flavanoids, Steroids, Terpenes, Proteins.

Table No 3: Results Of Phytochemical Screening

Phytochemical test	Test used	ASE	AME	PBE	MAE
Alkaloids	Dragendroff's test	+	+	+	+
	Mayers test	+	+	+	+
	Wagners test	+	+	+	+
Glycosides	Keller – Kilani test	+	+	+	+
Flavonoids	Shinoda test	+	+	+	+
Steroids	Liebermann-buchard test	+	+	+	+
Terpenes	Salkowaski test	+	+	+	+
Proteins	Ninhydrin test	+	+	+	+

Anti- bacterial susceptibility test:

After 24 hours of incubation at 37°C, Different concentrations of ASE, AME, PBE, MAE produced different circular and clear inhibition zones against *Staphylococcus aureus* and *Escherichia coli*, all four extracts have showed a significant increase in zone of inhibition as the concentration increased.

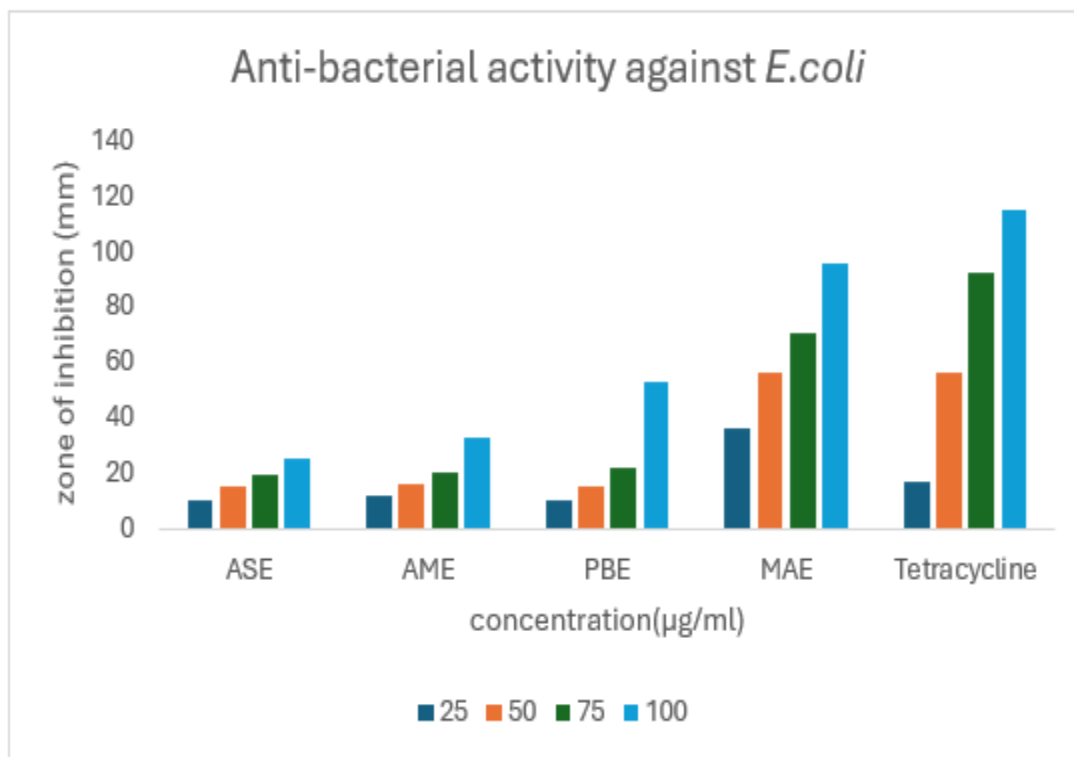
Escherichia coli,

ASE produced zone of inhibition of 10mm,15mm,19mm,25mm for 25,50,75,100 ug/ml respectively, AME produced zone of inhibition of 12mm,16mm,20mm,33mm for 25,50,75,100 ug/ml respectively, PBE produced zone of inhibition of 10mm,15mm,22mm,53mm, for 25,50,75,100 ug/ml respectively, MAE produced zone of inhibition of 41mm,62mm,83mm, and 102mm, respectively, and the standard Tetracycline produced zone of inhibition of 17mm,56mm,92mm,115mm, respectively.



Table no 4: zone of inhibition of ASE, AME, PBE, MAE against *E. coli*

Concentration (ug/ml)	Zone of inhibition (mm)				
	ASE	AME	PBE	MAE	Tetracycline
25	10	12	10	36	17
50	15	16	15	56	56
75	19	20	22	70	92
100	25	33	53	95	115

**Fig no 5: Antibacterial activity of ASE, AME, PBE, MAE against *E.coli******Staphylococcus aureus***

ASE produced zone of inhibition of 16mm,23mm,27mm,34mm for 25,50,75,100 ug/ml respectively, AME produced zone of inhibition of 15mm,21mm,24mm,38mm for 25,50,75,100 ug/ml respectively, PBE produced zone of inhibition of

16mm,22mm,29mm,68mm, for 25,50,75,100 ug/ml respectively, MAE produced zone of inhibition of 41mm,62mm,83mm, and 102mm, for 25,50,75,100 ug/ml respectively, and the standard Tetracycline produced zone of inhibition of 24mm,63mm,102mm,121mm, for 25,50,75,100 ug/ml respectively.

Table no 5: zone of inhibition of ASE, AME, PBE, MAE against *S. aureus*

Concentration (ug/ml)	Zone of inhibition (mm)				
	ASE	AME	PBE	MAE	Tetracycline
25	16	15	16	41	24
50	23	21	22	62	63
75	27	24	29	83	102
100	34	38	68	102	121

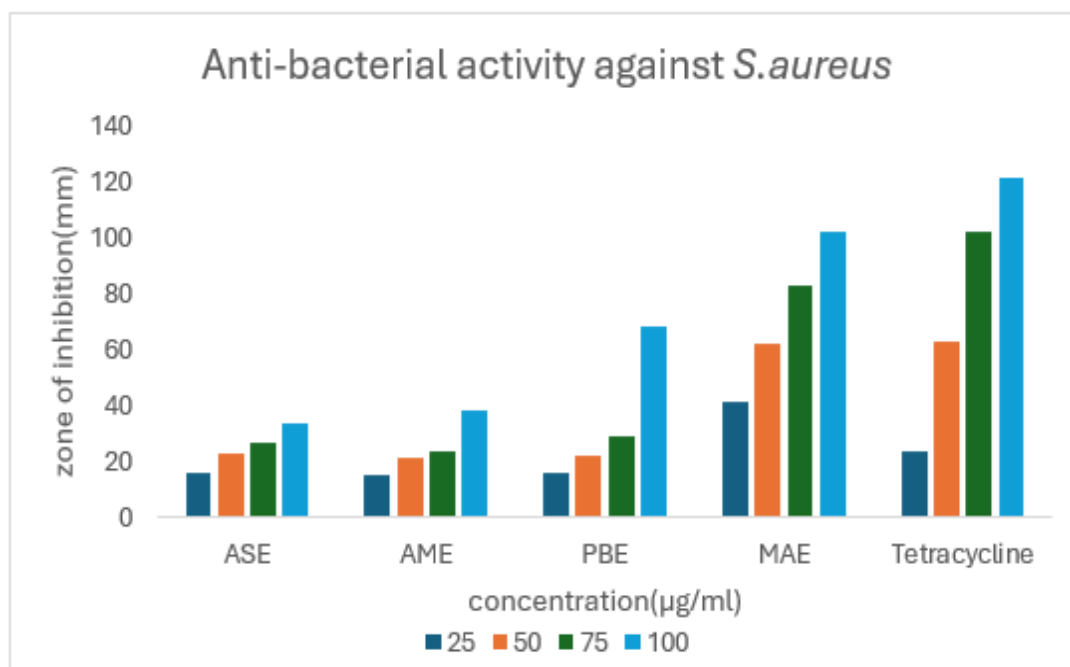


Fig no 6: Antibacterial activity of ASE, AME, PBE, MAE against *S.aureus*

CONCLUSION:

Anti-microbial resistance developed by microorganisms against anti-microbial agents can be seen as a new threat, which leads to the development of superbugs. In our present study, we studied the anti-bacterial properties of four plants *Annona squamosa*, *Aegle mermelos*, *Piper betle*, *Mentha averensis*, which are commonly found in Korangi. From the results of anti-microbial study we conclude that all the four selected plants have significant effect on inhibition of microorganism growth, maximum inhibition was observed for *Mentha averensis* with maximum zone of inhibition of 95mm for *E.coli* and 102 mm for *S.aureus* at 100µg/ml, Hence further research on the above plants can give potential anti-microbial agents.

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