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Comprehensive Analysis of Antioxidant and Antimicrobial Properties of Aegle Marmelos Leaf Extracts

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Abstract: ***In the existing study, we carried out an antimicrobial activity of serial water, ethanol, methanol, and acetone extract from leaves of Aegle marmelos investigated against bacterial species. All the extracts exhibited antimicrobial activity with zones of inhibition ranging from 8 to 13mm against bacteria Staphylococcus aureus and antimicrobial activity was not found against Escherichia coli. The physical parameters were determined as pH, total ash, acid soluble ash, acid insoluble ash value, water extract, ethanol extract, methanol extract, and acetone extract. phytochemical screening revealed the presence of phenols, carbohydrates, alkaloids, tannins, flavonoids, and saponins in extracts. The FTIR spectra of water extract, methanol extract, ethanol extract, and acetone extract were also performed. The FTIR spectra analysis gives an idea about the different functional groups present in this sample and it can be isolated and can be used as the active components of this natural plant for further drug preparation. The free radical scavenging activity of different extracts was evaluated by using the DPPH method. The free radical scavenging effect was observed in leaves with IC_{50} = 28.45µg ml^{-1}. The ability of the leaf extracts of Aegle marmelos to inhibit the growth of bacteria is an indication of its broad-spectrum antimicrobial activity which could be a potential source for the development of novel bioactive antimicrobial agents.***

Keywords: Aegle marmelos, Antioxident activity,Antimicrobial activity, FTIR analysis, Phytochemical screening

1. Introduction

Bael, *Aegle marmelos* is one of the medicinally treasured tree species [1]. Medicinal plants act as an indigenous source of new compounds possessing therapeutic value and can also be used in drug development. 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 [2]-[5]. 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 [6]-[13]. Plants continue to be a major source of commercially consumed drugs. Even many synthetic drugs have their origin in natural plant products. The trend of using natural products has increased in recent years and active plant extracts are frequently screened for new drug discoveries [14]. *Aegle marmelos* belongs to the family *Rutaceae*, commonly known as bael (Hindi). It is found throughout India and is known from pre-historic times. *Aegle marmelos* has been used from time immemorial in traditional systems of medicine for relieving constipation, diarrhea, dysentery, peptic ulcer, and respiratory infections [15]. Several studies on different parts of *Aegle marmelos* showed that the plant possesses antidiarrhoeal [16], antidiabetic [17], anti-inflammatory, antipyretic, analgesic [18], anticancer [19], radioprotective [20], and antimicrobial activities [21]-[22]. Limited information is available regarding the antimicrobial activity of *Aegle marmelos* leaves; therefore, the present study is carried out to investigate the antimicrobial activity of serial extracts from leaves of *Aegle marmelos* against various bacterial and fungal species. Preliminary phytochemical studies of these extracts are also undertaken to find out whether bioactive compounds have antimicrobial activity.

2. Materials and Methods

The leaves of Aegle marmelos were collected from the botanical garden of GVISH Amravati. It was washed 2-3 times with distilled water and shade dried. The dried leaves were powdered in a mixer grinder and kept in a plastic bag until it was analyzed.

Preparation of extracts

Water extraction: The aqueous extracts of the sample were prepared by boiling 5gm of finely powdered leaves in 100 ml of double distilled water at 900C for one hour while stirring using a magnetic stirrer at 900rpm. The extract was then cooled to room temperature and filtered using Whatman No1 filter paper and stored in a refrigerator at 40C for further experimental use.

Ethanol, Methanol, and Acetone Extracts: 10gm of the sample was kept in the three beakers 100ml of each solvent was added in each beaker respectively. The sample was socked in solvent for 12hr at room temperature. It is then filtered. The excessive solvent was evaporated. The extract was weighed and calculated in percentage.

Determination of pH range: 5gm of the sample was weighted and immersed in 100ml of water in a beaker and the pH of the formulation was determined using a calibrated pH meter.

Determination of Total ash: Accurately 5gm of the sample was taken in finely clean and previously weighed silica crucible and ignited for 3-4 hr with gradually increasing in temperature up to 500oC. After ignition of leaves and crucible were cooled in a desiccator and weighed as total ash. The ash was used to determine the acid-soluble ash and acid-insoluble ash.

Determination of Acid insoluble ash: The ash was dissolved in 2N hydrochloric acid in the beaker. Stirred well for the digestion of ash and filtered through Whatman filter paper. The residue remains after filtration is ignited in a clean silica crucible by gradually increasing the temperature up to 500oC. The crucible with the residue was cooled in desiccators and weighed. The residue that remains after the ignition was calculated as acid-insoluble ash in percentage. From this calculation, the acid-insoluble ash was calculated by taking the difference between the Total ash & acid insoluble ash in percentage.

Preliminary phytochemical screening: The aqueous, methanol, ethanol, and acetone extracts were used for preliminary phytochemical analysis using standard procedures [23, 24].

a) Test for alkaloids:

Wagner's test: About 10 mg of extract was taken and a few drops of Wagner's reagent were added and the formation of a reddish brown precipitate indicates the presence of alkaloids.

b) Test for Flavonoids: Shinoda Test: 10mg of extract was added to a pinch of magnesium turnings and 1-2 drops of concentrated hydrochloric acid was added. The formation of pink color indicates the presence of Flavonoids.

c) Test for Phenols & Tannins:

Ferric chloride test: 5mg of extract was taken and 0.5 ml of 5% ferric chloride was added. The development of bluish color indicates the presence of tannins.

d) Test for steroids and sterols:

Salkowski's test: 5mg of extract was dissolved in 2 ml of chloroform and an equal volume of concentrated sulphuric acid was added along the sides of the test tube. The upper layer turns red and the lower layer turns yellow with green fluorescence, indicating the presence of the steroids and sterols compound in the extract.

e) Test for carbohydrates: Fehling's test: 5ml of Fehling's solution is a water bath. The formation of yellow or rest for precipitate indicates the presence of reducing power.

f) Test for Saponins: **Foam test:** 0.5 mg of extract was diluted with 20 ml distilled water and shaken well in a graduated cylinder for 15 min. The formation of foam to a length of 1cm indicated the presence of saponins

g) Test for Glycosides: Glycoside test: 0.5 mg of extract was dissolved in 1 ml of water and then aqueous NaOH solution was added. The formation of yellow color indicates the presence of glycosides.

Figure 1: Extracts by using different solvents. (ethanol, methanol, acetone, water

3. Result and Discussion

Table 1: Determination of pH of Aegle marmelos Leaves

S. no.	Parameter	Result
1	pH meter value	5.59

Table 2: Determination of different ash value of Aegle marmelos leaves

S. No.	Parameter	Result
1	Total ash value	3.7%
2	Acid soluble ash value	1.2%
3	Acid insoluble ash value	2.3%

Table 3: Quantitative determination of Aegle marmelos leaves extract in various extract

Plant constituent	Water extract	Ethanol extract	Methanol extract	Acetone extract	Name of test
Alkaloids	+	+	+	+	Wagner's test
Phenols, tannins	+	+	+	+	Ferric chloride test
Steroids, sterols	–	–	–	–	Salkowaskis test
Carbohydrates	+	+	+	+	Benedict test
saponins	+	_	_	_	Foam test
glycosides	_	_	_	_	Glycosides test

Table 4: Preliminery phytochemical screening of A. marmelos[positive+,negative-]

Sr.no.	Parameter	Result
1.	Water extract	22.5%
2.	Ethanol extract	18.09%
3.	Methanol extract	10.01%
4.	Acetone extract	5.79%

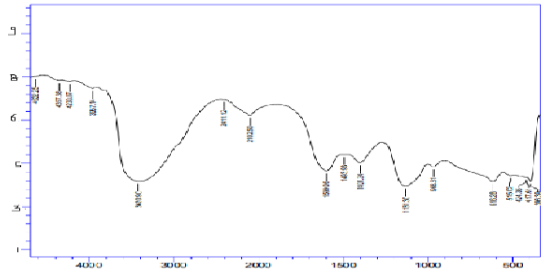

Figure 2: FTIR spectra of water extract of A. marmelos leaves

Table 5: FTIR spectra analysis of water extract of A. marmelos leaves

S. No	Frequency cm^{-1}	Bond	Functional group
1.	3420.90	O-H stretch	Carboxylic acid
2.	2411.12	C-H stretch medium	Alkane
3.	2102.5	C≡C stretch medium	Alkynes
4.	1599.06	C=C stretch	Cyclic alkenes
5.	1493.13	C-H stretch bending	Methyl group
6.	1131.3	C-O stretch medium	Alcohol
7.	968.31	C-O stretch medium	Alcohol
8.	616.28	C-Br stretching	Halogen group
9.	515.02	C-Br stretching	Halogen group
10.	424.36	C-I stretching	Halogen group
11.	406.23	C-I stretching	Halogen group

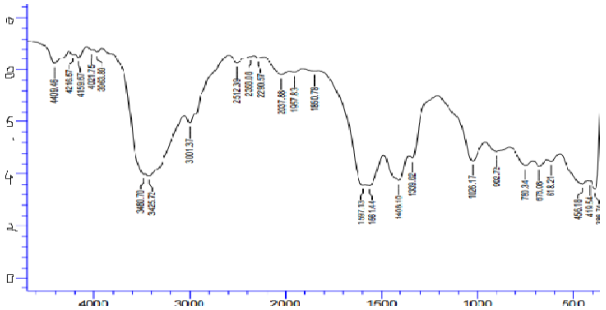

Figure 3: FTIR spectra of ethanol extract of A. marmelos leaves

Table 6: FTIR spectra analysis of ethanol extract of A. marmelos leaves

S. no	Frequency cm^{-1}	Bond	Functional group
1.	3643.89	O-H stretch	Carboxylic acid
2.	2812.39	C-H stretch medium	Alkane
3.	2358.08	C-H stretch medium	Alkane
4.	2290.47	C-H stretch medium	Alkane
5.	1850.18	C=O stretch medium	Carbonyl group
6.	1561.44	C-H stretch bending	Methyl group
7.	1468.01	C-H stretch bending	Methyl group
8.	1339.62	C-H stretch bending	Methyl group
9.	1026.17	C-O stretch medium	Alcohol
10.	962.72	C-Br stretching	Halogen compound
11.	750.34	C-Br stretching	Halogen compound

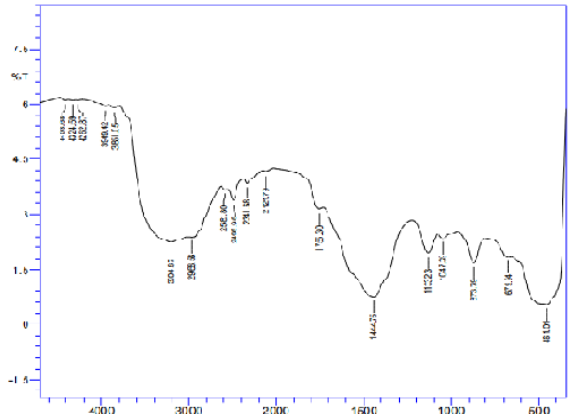

Figure 4: FTIR spectra of methanol extract of A. marmelos leaves

Table 7: FTIR spectra analysis of methanol extract of A. marmelos leaves

S.no.	Frequency cm^{-1}	Bond	Functional group
1.	2968.57	C-H stretch medium	Alkane
2.	2582.8	C-H stretch medium	Alkane
3.	1755.3	C=O stretch medium	Carbonyl group
4.	1444.75	C=C stretch medium	Aromatic ring
5.	1243.16	C-O stretch bending	Alcohol
6.	1047.59	C-O stretch medium	Alcohol
7.	873.79	C-Br stretching	Halogen compound
8.	679.94	C-Br stretching	Halogen compound
9.	461.01	C-I stretching	Halogen compound

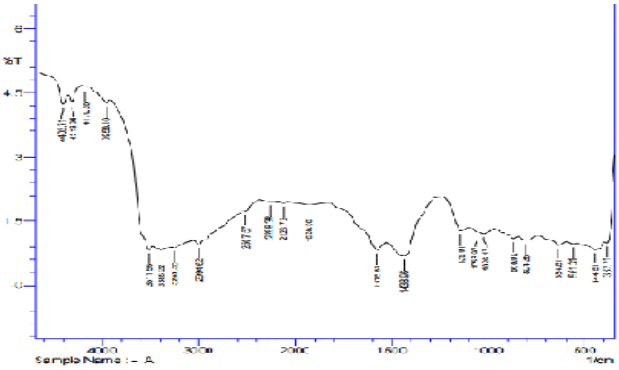

Figure 5: FTIR spectra of Acetone extract of A. marmelos leaves

Table 8: FTIR spectra analysis of Acetone extract of A. marmelos leaves

S.no.	Frequency cm^{-1}	Bond	Functional group
1.	2994.62	C-H stretch medium	Alkane
2.	2507.57	C≡C stretch medium	Alkynes
3.	1928.9	C=O stretch medium	Carbonyl group
4.	1438.96	C=C stretch strong	Cyclic alkenes
5.	1020.17	C-O stretch bending	Alcohol
6.	804.32	C-Br stretching	Halogen compound
7.	561.31	C-I stretching	Halogen compound

Antimicrobial activity

The extract mentioned above were tested against two pathogenic bacterial strains one gram-positive bacteria S.aureus and one gram-negative bacteria E.coli All the extracts exhibited antimicrobial activity with zones of inhibition ranging from 8 to 13mm against bacteria Staphylococcus aureus and antimicrobial activity was not found against Escherichia coli.

Table 8: Zone of inhibition by serial extracts

	Zone of inhibition				
Test microorganism	Water extract	Ethanol extract	Methanol extract	Acetone extract	standard
Staphylococcus aureus	12.5mm	11mm	9.1mm	8mm	14mm
Escherichia Coli	-	-	-	-	13mm

Antioxidant activity

Antioxidants are significant in the prevention of human illness and may function as free radical scavengers, complexes of prooxidant metals, reducing agents, and quenchers of singlet oxygen formation. Free radicals possess the ability to reduce the oxidative damage associated with many diseases including neurodegenerative diseases, cancer, and cardiovascular diseases. Antioxidants through their scavenging power are useful for the management of these diseases.DPPH stable free radical method is an easy, rapid, and sensitive way to survey the antioxidant activity of specific compounds or plant extracts.

Table 9: Percent inhibition by serial extracts.(antioxidant assay)

Leaves extract	% inhibition
Water extract	63%
Ethanol extract	69.39%
Methanol extract	39%
Acetone extract	23.9%

4. Conclusion

The A. marmelos leaves extract was tested for various physical properties such as pH, total ash, acid soluble ash, acid insoluble ash, water extract, ethanol extract, methanol extract, and acetone extract were determined. The pH was observed 6.04. the total ash content in the sample indicates the amount of inorganic oxides present in it. the total ash found in A. marmelos leaves was 3.7%this may be because we have taken its leaves for estimation, the acid soluble ash was found to be 1.2% and the acid insoluble ash value was 2.3%. The water extract of leaves of this sample shows that maximum water extract is obtained at 22.5% and minimum in Acetone 5.79%.waterextract<ethanol extract<methanol extract< acetone extract.Phytochemicals are plant nutrient that acts as secondary metabolized.FTIR spectra analysis showed different peaks of chemical constituents present in it. This spectra analysis gives an idea about the different functional groups present in this sample and it can isolate the active components of this natural plant for further drug preparation. The free radical scavenging activity of the different extracts was evaluated by using the DPPH method. The free radical scavenging effect was observed in leaves with IC_{50} = 28.45µg ml^{-1}. All the extracts exhibited antimicrobial activity with zones of inhibition ranging from 8 to 13mm against bacteria Staphylococcus aureus and antimicrobial activity was not found against Escherichia coli.

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