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***In-vitro* evaluation of antimicrobial and antioxidant activity of *Embelica officinalis* extract on different strains on gram positive and negative bacteria**

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Abstract--The present study was carried out to evaluate the *in vitro* antimicrobial and antioxidant activity of *Emblca officinalis* extract powder. The antimicrobial activity was assessed against gram positive and gram negative bacteria namely *E.coli*, *Pseudomonas fluoresces*, *Bacillus subtilis*, *S.aureus*, by agar well diffusion method. Quantitative antimicrobial susceptibility testing method, i.e., determination of minimum inhibitory concentration (MIC) by serial broth dilution was carried out on the different bacterial strains. The diameter of the zone of inhibition (ZOI) for the strains tested against was recorded and compared with the standard (Gentamicin, in case of bacteria). The results of the present study also revealed that Amla extract powder can scavenge free radicals or reactive oxygen species under *in vitro* conditions. The elimination of hydrogen peroxide by Amla powder may be attributed to phenolic compounds. The total phenolic content in the *Emblca officinalis* extract powder measured by the Folin Ciocalteau reagent in terms of gallic acid equivalents (GAE) was found to be 390.8mg/g. The possible reason for antibacterial activity of Amla extract powder might be the presence of tannins in the fruits. Therefore, Amla extract powder has great potential as an antimicrobial agent and can be used to treat infectious diseases caused by resistant microorganisms. The presence of phenolic compounds and flavonoids may be attributed to the antioxidant activity of the plant. Thus, it can be concluded that the *Emblca officinalis* extract powder has high antibacterial and antioxidant activity and can be further explored for the isolation of its bioactive compounds.

Keywords---*Emblica officinalis*, antimicrobial activity, antioxidant activity, Disk diffusion method.

Introduction

There is evidence that plants were rooted in the soil long before humans walked upon it and it is believed that God endowed plants with the resources necessary for the life of humans and animals long before he created these beings [1]. There is little doubt that herbal therapy is one of the earliest and oldest forms of human healing. The use of herbal medicine as a treatment for a variety of illnesses dates back a significant amount of time.

Most people in developing countries still use herbal remedies to cure their illnesses because they are more readily available, less expensive and have less adverse effects than allopathic pharmaceuticals, according to the World Health Organization (WHO). In the Indian Materia Medica, there are almost two thousand natural medications that have been documented to have varied pharmacological properties of these, approximately 1600 are derived from plant life. Herbal medicines have been the foundation of the traditional system of medicine for millennia and are the foundation of contemporary pharmacology (www.herbalremediesinfo.com/history-of-herbal-medicine.html). In spite of the fact that science has been working, on and off, since the 1880s roughly, to isolating the active chemicals that are present in herbal remedies, the list continues to expand [2]. Amla, or *Emblica officinalis*, is a native plant to India. It is often referred to as "Indian gooseberry." Amla has long been regarded as a staunch member of the *Euphorbiaceae* family, having literally hundreds of applications. Modern pharmacopoeia includes monographs on crude plant preparations. Modern isolation techniques indicate novel plant medications are usually purified rather than galenical formulations. As a result of the growing interest in herbal medicine, which depends mainly on plant-based sources rather than synthetic medications, there has been a resurgence in pure compound use. In response to the growing popularity of herbal medicines, the editor of Journal of Natural Products, 1999, notes that new articles reporting robust scientific investigations are requested. Undoubtedly, there are many kinds of plant in the plant world that contain medicinally valuable compounds that have yet to be identified; enormous numbers of plants were continually being examined for their potential bioactivity [3].

Recognizing that antibiotics have a finite lifespan, global spending on new anti-infectives has increased [4]. Since 1926, scientists have studied plants' antimicrobial properties. Numerous research groups are now conducting studies in this field of biology, with some focusing on drug-resistant bacterial species in particular [5,6]. The goal of this research is to see if extract powder made from *Emblica officinalis* fruits has antibacterial and antioxidant capabilities.

Material and Method

Chemicals

Mueller Hinton Broth (Himedia M391-100G), Mueller Hinton Agar No. 2 (Himedia M1084-500G), Mueller Hinton Agar, 2 percent Glucose with methylene blue (Himedia M1825 - 100G), Gentamicin (Product Code: TC026), Folin Ciocalteu reagent, sodium carbonate, Gallic acid, and Sodium hydroxide are the components that make up the Mueller Hinton Medium. pH-buffer saline, H₂O₂, Dragendorff's, Mayer's, Wagner's, -naphthol, Ferric chloride, Magnesium ribbon, Hydrochloric acid.

Phytochemical screening

The following are the recommended standard protocols that were followed in order to carry out the preliminary phytochemical screening [7-9].

Detection of carbohydrates: Before being passed through the filter, each of the extracts was first mixed with 5 milliliters of distilled water. Filtrate carbohydrates were analyzed.

Detection of Phenolic Compounds:

Ferric Chloride Test: In a test tube, the extract was treated by adding three to four drops of ferric chloride solution. Blue-black precipitate denotes phenolic chemicals.

Detection of Tannins:

Gelatin Test: A gelatin solution with sodium chloride and a concentration of one percent was added to the extract. Tannins form a white precipitate.

Determination Of Ash Content

When a sample is torched under the conditions indicated in the individual monograph, the Ash Limit tests are used to determine how much residual substance is present.

Determination Of Acid Insoluble Ash

The acid insoluble ash limit test are utilized for the purpose of determining the maximum amount of ash that is resistant to dissolving in diluted hydrochloric acid.

Determination Of pH

After careful weighing, 0.5 grams of amla powder were dissolved in 10 milliliters of purified water. The solution was filtered and the filtrate collected in a beaker. The pH of the 5% solution was read by the pH meter. The instrument was washed properly after usage.

Loss On Drying

It is used to determine how much water and other volatile substances are lost when a sample is dried in a certain manner. "Not more than 0.50 percent (105 °C, 3 hours)" in the Monographs means that when drying 1 to 2 g of the sample at 105 °C for 3 hours and accurately weighing it, the sample doesn't lose more than 0.50 percent of its weight. Also, a specification like "not more than 0.50 percent

(0.5 g, not more than 1.3kPa, 24 hours)" means that the sample loses no more than 0.50 percent of its weight when about 0.5 g of it is placed in a desiccator with silica gel as the desiccant and dried under pressure at 1.3kPa or less for 24 hours.

Heavy Metal Analysis by Atomic Absorption Spectroscopy

Absorption spectroscopy is a Spectro analytical method for determining chemical elements via the absorption of light by gaseous atoms.

Antibacterial Activity

Serial dilution method for the determination of the minimum inhibitory concentration. Determination of the maximum bactericidal concentration.

Evaluation of Antioxidant Activity

Total Phenolic Content

0.2N of Folin Ciocalteu reagent was prepared by pipetting 1ml of the same and diluting it 10 times with distilled water.

Preparation of sodium carbonate solution: 0.75 grams of sodium carbonate were dissolved in 10 milliliters of distilled water to make the sodium carbonate solution. Preparation of standard gallic acid: The preparation of the standard stock solution consisted of dissolving 5 mg of gallic acid into 10 ml of distilled water, which resulted in a final concentration of 500 g/ml.

Preparation of Sample: For the preparation of the sample, 500 mg of amla powder was dissolved in 10 ml of distilled water [10].

Hydrogen peroxide scavenging activity

Preparation of Phosphate Buffer Saline (PBS, pH 7.4): Dissolving 8 grams of sodium chloride, 0.2 grams of potassium chloride, 1.44 grams of sodium bicarbonate, and 0.24 grams of potassium phosphate in 800 milliliters of distilled water yielded phosphate buffer saline. In order to get the solution to a pH of 7.4, HCl was used to raise the volume to 1000 ml [11,12].

Statistical Analysis

The mean and standard deviation are used to express all data. A one-way analysis of variance was followed by a t-test. It was judged significant when the difference was less than 0.05.

Results & Discussion

Phytochemical screening

Table 1. Initial phytochemical screening tests performed on amla extract powder

S.No	Family of Compound	Test performed	Result
1.	Alkaloids	Dragendorff test; Mayer test Wagner's test	+ve +ve +ve
2.	Carbohydrates	Molisch's test	+ve
3.	Phenolic Compounds	Ferric Chloride test	+ve

4.	Tannins	Gelatin test	+ve
5.	Saponins	Froth test Foam test	+ve +ve
6.	Flavonoids	Ferric chloride Shinoda test	+ve +ve
7.	Proteins and Aminoacids	Ninhydrin test Xanthoproteic test	+ve +ve
8.	Steroids	Lieburmann Burchard's test	-ve

Physiochemical Evaluation

Table 2. Physiochemical evaluation of Amla extract powder

Sl No	Particulars	Content in sample
1	Total Ash Acid Insoluble	3.65 % w/w 0.85% w/w 3.61
2	pH (5% solution)	6.3% w/w
3	Loss on drying	3.65 % w/w 0.85% w/w 3.61
4	Total Ash Acid Insoluble	6.3% w/w

Instrumental Analysis

Heavy-Metal Analysis by Atomic Absorption Spectroscopy

Table 3. Heavy metal content in Amla powder

Sl No	Heavy metal	Content in sample	Limits	Protocol
1	Lead	1.15 ppm	<10 ppm	By AAS
2	Cadmium	0.02 ppm	<2 ppm	By AAS
3	Arsenic	0.73 ppb	<2 ppm	As per USP

The heavy metal content in the Amla extract powder was found to fall within the limits given by USP/IP.

The standard calibration curves of lead, cadmium and arsenic are represented in the figures below:

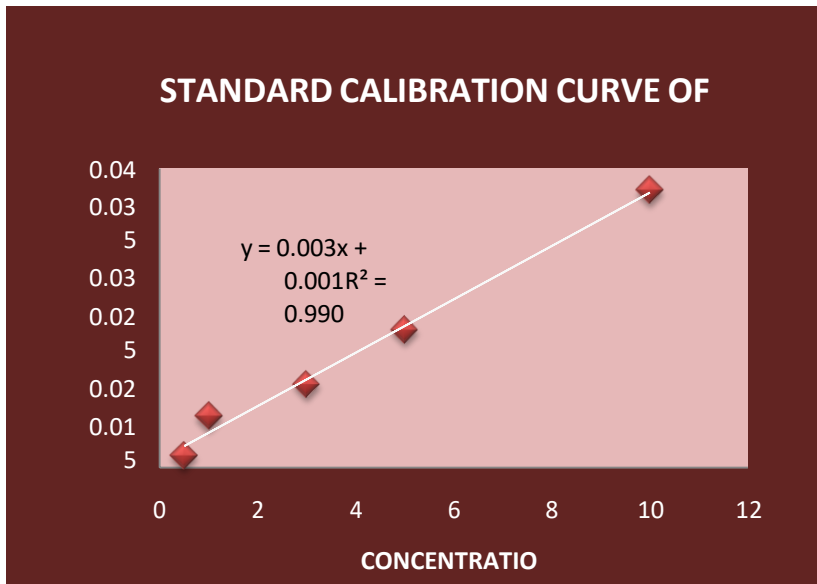


Fig. 1. Graph showing the standard calibration curve of Pb

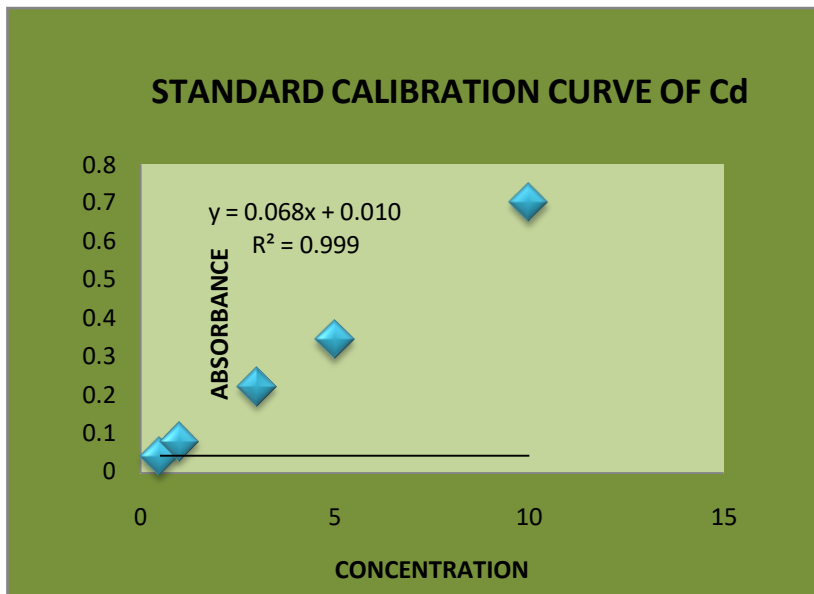


Fig. 2. Graph showing the standard calibration curve of Cd

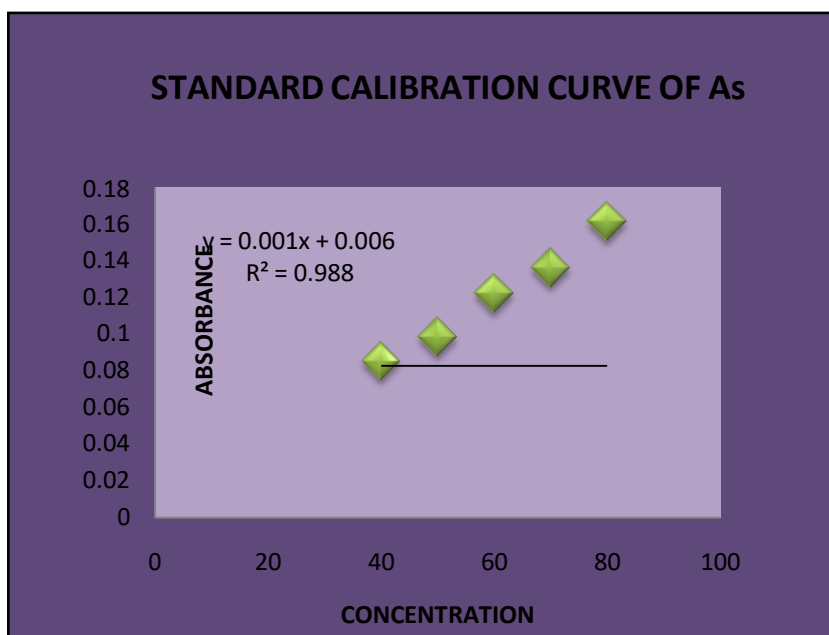


Fig. 3. Graph showing the standard calibration curve of as

Antimicrobial Activity

Antibacterial activity: The powder made from *Emblica officinalis* was tested against four different types of bacteria, including *E. coli*, *S. aureus*, *Pseudomonas fluorescens*, and *Bacillus subtilis*. It inhibited *Bacillus subtilis* more than *E. coli*. Table 4 and Figure 4 exhibit antibacterial activity assessed in the millimeter zone of inhibition. The amla extract powder has dose-dependent ZOI. Concentration raised ZOI levels. Gentamicin (10g/ml) was the standard antibacterial agent and demonstrated ZOI values against all microorganisms tested. Amla extract powder can be used to treat infectious disorders caused by resistant germs. Tannins in Amla fruits may explain its antibacterial effect.

Tables 5 and 6 show the MIC and MBC of the Amla extract powder against the tested bacterial strains. Low-activity antimicrobials have a high MIC and MBC. High-potency antimicrobials have low MIC and MBC.

Zone Of Inhibition

Table 4. Antibacterial activity of Amla powder against bacterial strains (Zone of Inhibition in mm)

Concentration (mg/ml)	Zone of Inhibition (in mm) [Mean ± SD]			
	<i>E.coli</i>	<i>S.aureus</i>	<i>Pseudomonas fluorescens</i>	<i>Bacillus subtilis</i>
0.5	6.0 ± 0.15	7 ± 0.10	6.5 ± 0.25	7 ± 0.30
1.0	7.5 ± 0.2	10 ± 0.21	10.5 ± 0.30	9 ± 0.20

1.5	8.5 ± 0.3	11 ± 0.25	12 ± 0.4	11.5 ± 0.1
2.0	9.5 ± 0.15	12 ± 0.21	13.5 ± 0.10	14 ± 0.35
Gentamicin (10 µg/ml)	15 ± 0.2	20 ± 0.3	20 ± 0.2	29 ± 0.21

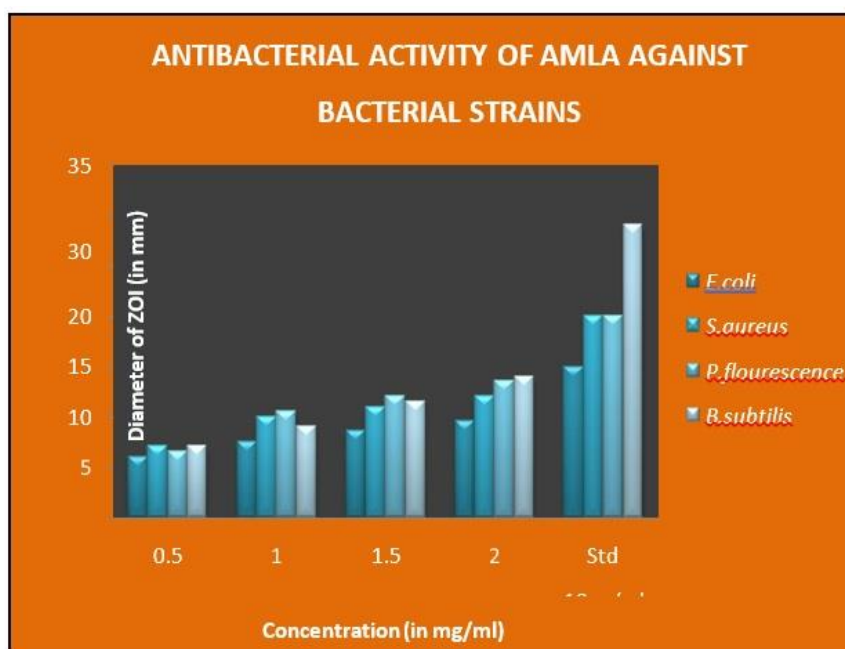


Fig. 4. Antibacterial activity of Amla against bacterial strains

Minimum Inhibitory Concentration (Mic)

Table 5. Antibacterial activity of Amla powder against bacterial strains (Minimum inhibitory concentration in mg/ml)

Bacterial species	<i>E.coli</i>	<i>S.aureus</i>	<i>Pseudomonas fluorescence</i>	<i>Bacillus subtilis</i>
MIC (mg/ml)	6.25	6.25	3.125	6.25

Minimum Bactericidal Concentration (Mbc)

Table 6. Antibacterial concentration of Amla powder against bacterial strains (Minimum Bactericidal Concentration in mg/ml)

Bacterial species	<i>E.coli</i>	<i>S.aureus</i>	<i>Pseudomonas fluorescence</i>	<i>Bacillus subtilis</i>
MBC (mg/ml)	25	Not determined	25	100

Antioxidant Activity Total Phenolic Content

The Folin-Ciocalteu reagent was used to test the total phenolic content in the Amla extract powder, and the total amount of gallic acid equivalents (GAE) was 390.8 mg/g.

Hydrogen Peroxide Scavenging Activity

Emblica officinalis extract powder could remove hydrogen peroxide in a way that depended on how much of it was there. Figure 5 shows the percentage reduction for *Emblica officinalis* powder and ascorbic acid. Under *In-vitro* settings, Amla extract powder is capable of scavenging free radicals and reactive oxygen species, according to the findings of the present study. Phenolic components may scavenge hydrogen peroxide. The plant's antioxidant phenolic chemicals and flavonoids are present.

Table 7. Percentage inhibition of H₂O₂ of Amla extract powder and Ascorbic acid

Sl No	Concentration (µg/ml)	% Reduction of Amla powder	% Reduction of ascorbic acid
1.	100	17.98 ± 0.48	38.37 ± 1.39
2.	200	41.36 ± 1.19	54.95 ± 0.69
3.	300	56.71 ± 0.59	67.11 ± 0.32
4.	400	77.24 ± 0.76	78.15 ± 0.27
5.	500	92.58 ± 0.79	95.25 ± 0.77

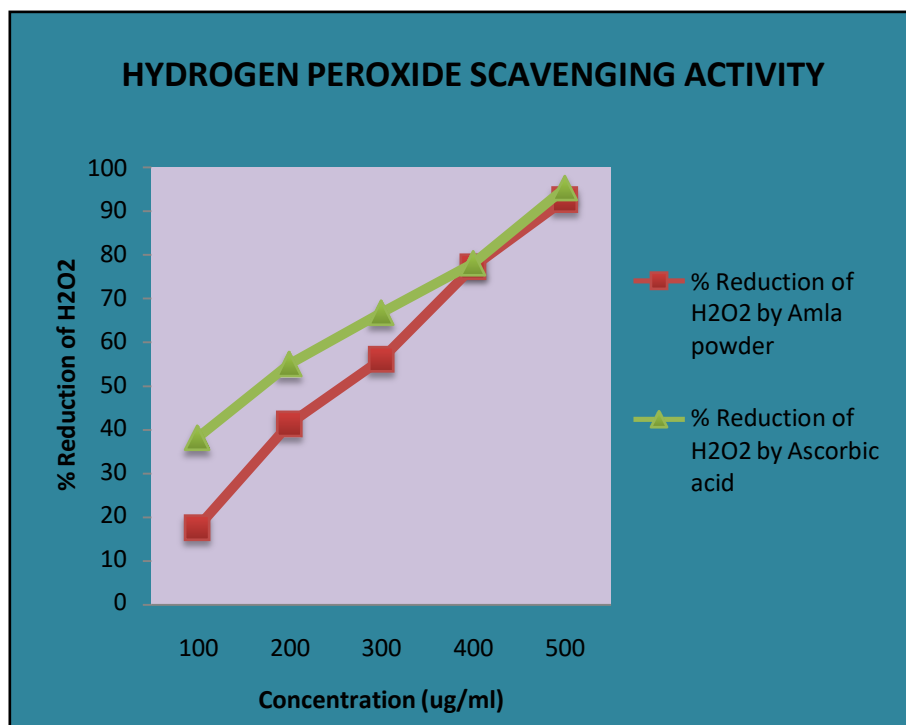


Fig.5. Hydrogen peroxide scavenging activity of Amla

Discussion

Naturally occurring substances can serve as good starting points for the development of new drugs because they have long been the foundation of conventional medicine. About 80% of the world's population depend on natural medicine for healthcare and plays a significant role with in remaining 20% system. WHO encourages, promotes, and facilitates herbal medicine use in poor countries for health projects. Using plants' natural products as models for new antibacterial drugs is a very important step to take, especially since bacterial resistance and multidrug resistance are problems that keep getting worse. *E. coli*, *S. aureus*, *Pseudomonas fluorescens*, and *Bacillus subtilis* were the four different strains of bacteria that were used in the study to investigate the antibacterial properties of amla extract powder. It inhibited *Bacillus subtilis* more than *E. coli*. The amla extract powder has dose-dependent ZOI. Concentration raised ZOI levels. Furthermore, as a standard antibacterial agent, gentamicin was used at a concentration of 10 micrograms per milliliter, and its ZOI values were positive against all kinds of bacteria that were examined.

The minimum inhibitory concentrations (MIC) of Amla extract powder against the tested bacterial strains are 6.25 mg/ml for *E.coli*, *S.aureus* and *Bacillus subtilis* and 3.125 mg/ml for *Pseudomonas flourescens*. Minimum bactericidal concentration (MBC) values of Amla extract powder for the different strains are 25 mg/ml for *E.coli* and *Pseudomonas flourescens* and 100 mg/ml for *Bacillus*

subtilis. Antimicrobial agents with low activity against an organism have a high MIC and MBC while highly active antimicrobial agents have a low MIC and MBC.

Herbs that are naturally astringent due to the presence of tannins in their make-up are frequently used in the treatment of intestinal conditions such as diarrhoea and dysentery. Antimicrobial tannins are well-documented. Many bacteria, fungi, yeasts, and viruses were suppressed by tannins. Phytophenols may give plants antioxidative properties. The phenolic chemicals and flavonoids found in plants are antioxidants, free radical scavengers, anti-inflammatory, and anticarcinogenic. Flavonoids are a polyphenolic substance with anti-inflammatory, anti-allergenic, antiviral, anti-aging, and anti-carcinogenic characteristics. Saponins are a useful component of plants. Saponins have antibacterial, antiprotozoal, and anticancer [13,14] properties. Natural antioxidants, especially phenolics, have been closely studied as possible therapeutic agents against neurological illnesses, cancer, diabetes, cardiovascular dysfunctions, inflammatory diseases, and aging. Medicinal effects of phenolics are attributed to their antioxidant activity, free radical scavenging, chelation of redox active metal ions, gene expression modification, and cell signaling pathways interaction. Folin-Ciocalteu reagent was used to assess Amla extract powder's phenolic content. Polyphenols have antioxidative capabilities due to its high reactivity as hydrogen or electron donors, their capacity to stabilize and delocalize unpaired electrons (chain-breaking function), and their ability to bind metal ions (termination of the Fenton reaction). Hydrogen peroxide, not a radical, contributes to oxidative stress. Biological systems may benefit from even low amounts of H_2O_2 . Iron complexes inside the cell can react with H_2O_2 *in vivo* to form highly reactive hydroxyl radicals, causing many of its harmful effects [15]. The phenolics in the extracts can give electrons in H_2O_2 , dissolving it to water. Ascorbic acid, which is a standard compound, was used to compare how well the powder could remove hydrogen peroxide. Amla extract powder scavenged hydrogen peroxide concentration-dependently. Ohwada et al. found a moderate association between the number of total phenolics in natural extracts and biological activity.

Amla extract powder contains carbohydrates, alkaloids, saponins, flavonoids, tannins, proteins, and amino acids. These physiologically active molecules boost Amla's actions. Secondary metabolites have diverse biological effects. Tannins bind irreversibly to proline-rich proteins, inhibiting cell protein synthesis. Tannins combine with proteins to produce the tanning effect, which is useful for treating inflammatory or ulcerated tissues. Tannin-rich herbs are astringent and used to treat diarrhoea and dysentery. Tannins have antibacterial properties. Tannins hinder bacteria, fungi, yeasts, and viruses. Plants' phenolic chemicals may be antioxidants. Phenolic chemicals and flavonoids found in plants are antioxidant, free radical scavengers, anti-inflammatory, anticarcinogenic, etc. Flavonoids are polyphenolic compounds with anti-inflammatory, anti-allergenic, anti-viral, anti-aging, and anti-carcinogenic characteristics. Saponins are helpful plant chemicals. Saponins are antibacterial, antiprotozoal, and anticancer [16].

A food's nutritive value can be thought of as an indicator of the contribution that it makes to the overall nutrient content in food. *Emblica officinalis* produces fresh or dried amla. Amla has a lot of glucose, pectins, gallic acid, ellagic acid, tannins, and vitamin C. Amla does have a very high nutritional value, is an

excellent liver tonic, a mild laxative, an astringent, and a diuretic. It is also the richest reported rich in vitamin C. Amla demonstrates efficacy against scurvy, and it has a very high anti-scorbutic potential.

Conclusion

Cold pressor extractor was used to extract the extract from the fruits of *Emblica officinalis*, which was then vacuum dried. The powder was subjected to physio-chemical evaluation such as pH, determination of ash and acid insoluble ash. The agar well diffusion method was used to test *in-vitro* antibacterial activity on many bacterial species, including *E.coli*, *S.aureus*, *Pseudomonas fluorescens*, and *Bacillus subtilis*. In addition, the quantitative antibiotic susceptibility testing method, also known as the persistence of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), was utilized in order to evaluate the degree to which the organisms were affected by the particular antimicrobial agent. *In-vitro* tests, utilizing industry standards, were used to investigate the antioxidant capacity of amla powder. Folin-ciocalteu method assessed total phenolic content. *In vitro* scavenging of hydrogen peroxide was another method used to assess the antioxidant activity of the sample.

The findings of this research indicate that the extract powder of *Emblica officinalis* possesses potent antibacterial and antioxidant activities, and the bioactive chemicals in its extract powder can be isolated and subjected to additional research if the researcher chooses to do so. Flavonoids, tannins, saponins, and phenolic chemicals are antibacterial and antioxidant.

Conflict of Interest: None

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