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In Vitro Antimicrobial Activity of Embelia Ribes Seeds

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ABSTRACT
Background: The commercially known as Baibidang, the fruits of the Embelia ribes plant are utilized to treat epilepsy, headaches, rhinitis, hemorrhage, and sleeplessness. Embelia ribes is beneficial for ringworm, intestinal worms, skin conditions, leprosy, itching, dental cavities, fever, headaches, and emaciation, among other conditions. Paste is used to treat skin infection. This plant contains several previously identified classes of components, like vilangin, christembine, quercitol, emblelina, and a volatile oil.
Objectives: Embelin demonstrated antifertility, analgesic, anti-inflammatory, antioxidant, and anticancer properties in animal tests. As a consequence, Embelia is chosen as the subject of the current antibiotic investigation. The current study is to analyze the ethanol extracts of Embelia ribes seeds physicochemically and investigate their antibacterial properties.
Materials and Methods: The antimicrobial activity against Staphylococcus aureus, Streptococcus faecalis, Streptococcus pyogenes, Escherichia coli, Salmonella typhi, Shigellaflexneri, Shigellaboydii, Shigellasonnei, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia, and E.
Results: Numerous bacterial and fungal pathogens that are assumed to be the cause of many common ailments were tested against the ethanolic extract. To evaluate the antibacterial activity of a plate, the zone where inhibition happens is assessed. The following boreholes were filled aseptically with standard solutions containing 500, 250, 125, 62.5, 31.25, and 15.625 mg/ml in 50 l, with ciproflaxin 10 g acting as a control sample.
Conclusion: Embelin demonstrated significant antibacterial properties against Staphylococcus aureus, Streptococcus pyogenes, Shigellaflexneri, S. sonnei, and Pseudomonas aeruginosa, in addition to to most against Salmonella typhi, S. boydii, as well as Proteus mirabilis.

Keywords: Embeliaribes seeds, Antimicrobial activity, Invitro, Against pathogens, Embelin, Phytochemical analysis, Minimum Inhibitory concentration.

INTRODUCTION

The Embelia ribes is the selected candidate for the present study and is well established for its wound healing and anti-inflammatory activity from ancient period, so as it aids in tooth decay its antimicrobial activity is also expected which creates an synergistic approach to wound care, hence it is selected as the candidate of interest for this study. Fruits have been utilized as an anthelmintic, astringent, alterative, tonic, and for the treatment of ascariasis, scorpion stings, and snake bites. The extract is helpful for fevers and chest as well as skin diseases. An underlying cause transfusion is employed to treat cough as well as diarrhoea. Antibiotic and antitubercular properties have been discovered in seeds.[2] Phytochemicals Plant-based substances known as phytochemicals have curative or disease-preventive qualities.[5-6] Phytochemical categories include polyphenols, tannins, phenolic acids, flavonoids, flavonols, isoflavones, lycopenes, epicatechins, anthocyanins, alkaloids, etc.[7] Quinones exhibit a wide spectrum of biological and physiological activities.[7] Recent studies have shown that these compounds, which plants make to protect themselves, also shield humans from disease. For instance, the isoflavonesgenistein and daidzein found in soy beans, soy milk, and tofu lower blood pressure and promote artery dilatation.[10] Garlic and onions include sulphides and thiols, which lower LDL cholesterol.[12] More than a thousand phytochemicals are known. About 100 novel plant-based medications were released onto the market by the USA between 1950 and 1970, including vinblastine, deserpidine, reseinnamine, reserpine, and vincristine, which are derived from higher plants.The anti-diabetic,[11] antibacterial,[12] and anticancer properties of oleanolic acid have been reported.[13]
The idea of receiving medical treatments via plant extracts is appealing to many individuals. Piper nigrum, Acoruscalamus, Piper betel, Curcuma longa, Thymus vulgaris, Cassia angustifolia, Glycyrrhizhaglabra,

Myristica fragrance, Allium cepa, Menthapiperita, Papaver somniferum, etc. are proven antimicrobial plants. Phenolic compounds like catechol, pyrogallol, cinnamic acid; flavones like abyssinone; flavonoids like chrysin; quinones like hypericin, Embelin; tannins like ellagitannin; alkaloids like berberine, terpenoids like menthol, artemisin, glycyrrhizhin; coumarins like warfarin are well known antimicrobial phytochemicals.[8] Finding out plants and phytochemicals with antibacterial activity is the scope of many researchers.

Embelia ribes, as well recognised as Baibidang, is a plant that grows with in Primulaceae family. It is commonly accessible across the whole of India. It really is largely regarded advantageous in a wide range of ailments in Ayurvedic medicine and Siddha.[3]Embelin, through specific, isolated from dried berries of Embelia ribes, seems to have a diverse variety of biological activities.[9]

The piney creeper bush Embelia ribes stems are brittle and movable. It was a herb known as vaividanga that was used medicinally for treating terete divisions. Simple, elliptic, alternating, and pointed at both ends are the characteristics of the leaves. The leaves measure 1.5 inches in width and 3 inches in length. Little, white flowers with tiny, three- to four-inch-long lobes are present. Fruits are small, resembling black pepper in color and ranging from reddish brown to black. Because of its natural order and appearance, it is often referred to as faked black pepper. We have found fruits in bunches. Fruits are found from the inside out, as their outermost covering is delicate. .[12-14]

MATERIALS AND METHODS

Phytochemical Investigation

General Materials

Chemicals and Solvents

The materials being used are of analytical reagent grade. For the extraction of the plant material, commercial-grade solvents were used. Commercial-grade ethanol was dried over quick lime and distilled.[10]

Instrument Details

All the inoculation and incubation were carried out is asceptic, sterile areas of chamber from bio-clean Air devices and Services. Perti Plates and all glassware is used from Borosil glass.

Plant parts

The vegetation material was gathered from Ahmednagar district in Maharashtra whilst it was flowering in August 2022.

PhytochemicalInvestigation

Extraction

The seedlings have been shade-grown and allowed to powder in a grinding machine. A 48-hour cold percolation process was used several times to produce 5 kg of rough powder. After being filtered and concentrated in a steam bath, the ethanol extract was vacuum-vacuumed and allowed to dry. In addition to conducting a phytochemical analysis of the entire alcohol extract (48 g), antibacterial activity was evaluated using all of these suitable alloquits.[10]

Antimicrobial Study

Chemicals and Glassware

The same solvents and chemical products used in this study were obtained from Merck Chemicals and were of analytical and science lab quality; Hi-Media supplied the media used in this investigation. Glassware made of Borosil glass has been used during the investigation. Growth conditions, glassware, and additional equipment have been employed after being sterilised for 15 minutes at 121°C and 15 lbs/sq. inch of pressure in an autoclave.[11]

METHODLOGY

Stock and Working Solutions of Plant Extract

A standard solution with a concentration of 1,000 mg/ml is created by carefully weighing one gram of Embelia ribes alcoholic extract and dispersing it in one milliliter of distilled water. From these standard solutions, replicates containing 500, 250, 125, 62.5, 31.25, and 15.625 mg/ml were created. Dilutions of 7.813, 3.906, 1.953, 0.977, and 0.488 mg/ml were used to find the minimal inhibitory concentration..[12]

Preparation of medium

Muller Hinton Inoculum has been made up of 300 g/l meat transfusion, 17.50 mg/l casein acid hydrolysate, 1.50 gm/l starch, and -17 g/l agar. Needs to weigh precisely 3.8 g of Mueller Hinton Agar as well as suspended it all in distilled water of 100 ml before sterilising it in an autoclave at 121°C at 15 lbs/sq.inch for 15 minutes.[14]

Sterilization

The testing microbiology laboratory was sterilized by fumigation with formaldehyde and potassium permanganate for 24 hr every month. The inoculum hood was cleaned with 95% ethanol and exposed to UV light for 20 min before every use. The digital weighing balance was also cleaned with 95% ethanol and surgical

gloves were used during the experiment for maintaining aseptic conditions. Test Organisms Organisms such as Staphylococcus aureus, Streptococcus pyogenes, Shigellaflexneri, S. sonneiand Pseudomonas aeruginosa; Salmonella typhi, S. boydiiand Proteus mirabilis were used for the study. ATCC cultures were grown from Christian Medical College, MTCC cultures from the Research center of Molecular Biology and biotechnology in Chandigarh, and NCIM cultures from the NCL, Pune. They were kept alive by sequential subculturing on nutrient-agar slants each quarter and incubating at 37°C for 18-24 hours. The cultures were kept cool by being kept in the fridge. All of the microbial tested organisms have been affirmed utilising different biochemical tests [14,15], as well as the fungal microbes have been affirmed to use a staining method.[16]

Antimicrobial activity

Utilizing a good dispersion technique, the antibacterial property was evaluated. A homogenous suspended sentence of microorganisms was prepared in 6 ml of saline and vigorously agitated to compare with McFarland's standards.[10] In order to achieve a density of 0.5 McFarland's unit, or a barium sulphate benchmark, the suspension was diluted with salt solution. The infected plates showed a difference in bacterial culture density even before a suspension was formed, after 15 minutes. The antibacterial property was ascertained using the well diffusion method. In 6 ml of saline, a homogeneous suspended sentence of microorganisms was ready and furiously stirred, to compare to McFarland's standards.[10] In order to achieve a density of 0.5 McFarland's unit, or a barium sulphate benchmark, the suspension was diluted with salt solution. The infected plates showed a difference in bacterial culture density even before a suspension was formed, after 15 minutes. In solidified Muller Hinton agar, 6 mm broad boreholes were made using a sterile plunger..[14-16]

Physico-Chemical Analysis

Determination of Loss on drying

In an accurately weighed flask, 4 g of drug was precisely weighed. It must have been left to dry for 5 hours at 105°C, cooled in desiccators, and weighed. Ongoing trying to dry as well as going to weigh at one-hour intervals until a consistent mass was obtained, i.e. two consecutive weighings after drying for 30 minutes and cooling for 30 minutes inside a desiccator had shown no and over 0.01 g variation.[12]

$$\text{Percentage of Loss on drying at 105°C} = \frac{\text{Loss in weight of the sample} \times 100}{\text{Weight of the sample taken}}$$

Determination of Total Ash

In an accurately weighed silica dish, burned approximately 2 g weighed accurately powder form of drug. The portion of ash was determined by calculating that use the air-dried drug as a reference.

$$\text{Percentage of Total ash} = \frac{\text{Weight of ash} \times 100}{\text{Weight of the sample}}$$

Determination of Water-soluble extractive

Five grams of the powdered oven dried medication were macerated with one hundred milliliters of distilled water inside a sealed flask for a duration of twenty-four hours. The mixture was shaken frequently for the first six hours and left to stand for eighteen hours. swiftly screened while preventing solvent loss. In a small, tared bowl, 25 mL of the filtrate was evaporated until dry. It was then dried at 105°C to a consistent mass and weighed. The medication was allowed to air dry before being used as a reference to assess the percentage of alcohol-soluble resource extraction..[12]

$$\text{Percentage of Water soluble extractive} = \frac{\text{Weight of the extract} \times 100 \times 100}{25 \times \text{weight of the sample taken}}$$

Determination of Alcohol soluble extractive

Macerated 5 g of the coarsely powdered air dried drug, with 100 ml of alcohol of the specified strength in a closed flask for twenty-four hours, shaking frequently during six hours and allowing to stand evaporated 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish for eighteen hours. Filtered rapidly, taking precautions against loss of solvent, and dried at 105°C, to constant weight and weighed. Calculated the percentage of alcohol-soluble extractive with reference to the air-dried drug.[16]

$$\text{Percentage of Alcohol soluble extractive} = \frac{\text{Weight of the extract} \times 100 \times 100}{25 \times \text{weight of the sample taken}}$$

Microbial Load

10 g of powdered sample was weighed accurately as well as suspended in nutrient broth before being diluted to 100 ml using the same form of media. The pH of the solution has been raised to 7.[16]

Total bacterial count

The sample was diluted to the concentration of 10^{-3}, 10^{-4} and 10^{-5} in Nutrient broth. One ml from different dilutions was placed on separate sterile petriplates containing presterilizedSoyabean casein digest agar medium.

The plates were kept at 37°C for 24 h before determining the number of colonial possessions that grew on them.[16]

Total fungal count

The sample preparation and dilution procedure was followed as above with Sabourauds dextrose agar instead of Soyabean casein digest agar and the plates were incubated at 25°C for 48 hr and the number of colonies found on the plate was recorded.[16]

Heavy Metal Analysis

An atomic absorption spectrophotometer has been used to approximate the concentrations of lead, cadmium, arsenic, and mercury—all of which are regarded as excessive metals—in a few chosen plants. This method is actually faster than other approaches and yields a higher proportion of component retrieval. The plant powder was dried at 105°C in an oven with air circulation. After carefully weighing five grams of the powdered sample, it was added to a conical flask and treated with five milliliters of nitric acid. Five milliliters of nitric acid were added to a flask without any sample to act as a blank. The flask's contents were placed on a glass plate for protection, and they evaporated to dryness at 80–100°C for just one hour on a plate that was heated electrically. After that, two milliliters of 30% hydrogen peroxide and 5 ml of nitric acid were added to the flask's contents, and the mixture was gently refluxed until the solutions became clear. Subsequently, the solutions were produced in 100 mL basic flasks and filtered through Whatman no. 42 filter paper. The lead and arsenic were both approximated using the hydride generator approach. The cold vapour approach has been used to estimate mercury, and the flame technique has been used to approximate cadmium. The standard curve created for each metal's stock solutions was examined to determine the identical concentration values of each metal.

RESULTS

Antimicrobial Study on Embelia ribes

The alcoholic extract of Embelia ribes fruits has been discovered to be effective against K. pneumoniae, E. coli, S. typhi, S. aureus, S. sonnei, St.pyogenes, P.aeruginosa, and S.flexneri in this study. The ethanolic extract was most effective against K. pneumoniae, E. coli, S. typhi, S. aureus, and S. sonnei, with P. aeruginosa and St. pyogenes being second and third, respectively. The activity against K. pneumoniae and S. sonnei was observed with a concentration of 500 mg/ml and also was discovered in lower doses. Other fungi and bacteria also weren't discovered to exist in the leaf extracts tested concentrations. The minimal inhibitory concentration (MIC) for S. aureus, S. typhi, and St. pyogenes was 125 (mg/ml); the MIC for K. pneumoniae and E. coli was 15.62 mg/ml.

The study's goal has been to see if an ethanolic extract of the entire plant of Embelia ribes has antimicrobial activity or otherwise. An alcoholic extract was tested against such a number of fungi and bacteria pathogens which are thought to be the source of many common bacterial infections. Table 1 displays the antibacterial activities and area of inhibitory activity results, and Figure 2 depicts the plates.

DISCUSSION

Klebsiella pneumonia is indeed a rod-shaped gram-negative microbes that has no motility and therefore is popularly separated from water, human and animal faeces, and soil. It infects surgical wounds, the urinary tract, and is discovered inside the respiratory system. It primarily affects the people who have underpinning illnesses such as diabetes or chronic lung diseases. Escherichia coli is a gram-negative rod-shaped microbes that can move. It's the most well-known participant of the Enterobacteriaceae family.It results in secondary infections.This is found in internal bleeding (inner abdominal inflammation), cholecystitis (gall bladder inflammation), septic wounds, and appendix abscesses. It usually causes relatively simple infectious diseases inside the urinary tract, however the extract has no effect on them. The minimal inhibitory concentration (mg/ml) against K. pneumonia was 62.5. E. coli, K. pneumonia, and S. typhimurium are rod-shaped gram - negative bacterium that cause gastrointestinal problems, urinary tract infections, and non-typhoidal fever. P. aeruginosa is a gram-negative microbes with such a rod shape and monopolar motility that causes diseases in plants, animal life, and living beings. It causes diseases of the respiratory tract, urinary bladder, burns, wounds, and other bloodstream infections. The drying loss of Embelia ribes collected in Ahmednagar has been determined by calculating to be 10.33%, indicating that the plant's humidity retention ability is greater.

The ash content value of Embelia ribes is 12.25%, indicating that now the seeds also contain a greater amount of inorganic materials, whereas the water soluble ash value of 6.63% indicates that nearly half of the total inorganic compound is soluble in water. When tried to compare to certain other plants, their own alkalinity values of 4.10 ml of 0.1N HCl/g of this would that the basicity of soluble in water inorganic content is significantly higher. The fruit's pH of 7.80 indicates that it is slightly basic in nature. The presence of highly polar groups is suggested by the 13.25% soluble in water extractive value. The ethanol soluble resource extraction value of 6.60% indicates it is lower than the soluble in water resource extraction value, and the higher loss dryer’s value may be the reason for the lower ethanol dissolved resource extraction.

Similarly, the higher overall ash content is the underlying cause. Even so, the total hot extraction values with

hexane and chloroform are less than 3%, indicating that now the plant is high in polar groups such as glycosides, tannins, and so on. As a result, the plant is safe to use as an internal medicine.

CONCLUSION

Embelia ribes fruit ethanolic extract has been effective against K. pneumoniae, E. coli, S. typhi, as well asSt.pyogenes. Klebsiella pneumonia is a gram-negative microbes with such a cylindrical shape and no motility. Embelin showed substantial antibacterial properties against Staphylococcus aureus, Streptococcus pyogenes, Shigellaflexneri, S. sonnei, as well as Pseudomonas aeruginosa, in addition to moderate activity against Salmonella typhi, S. boydii, and Proteus mirabilis. As just a result, the plant is safe to be used as an internal medicine.

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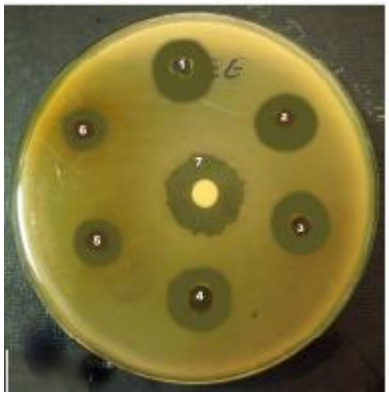
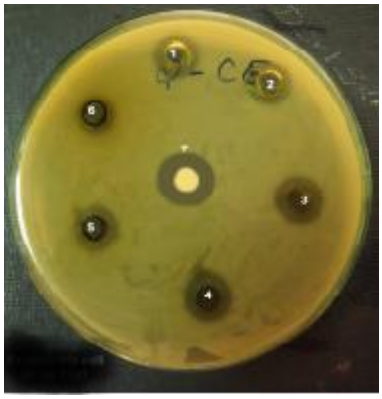
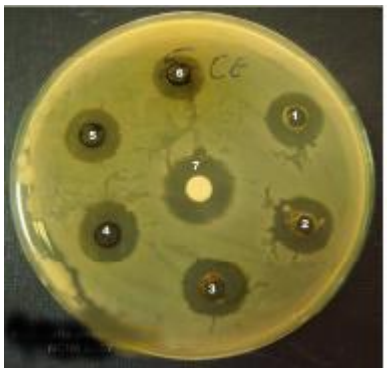
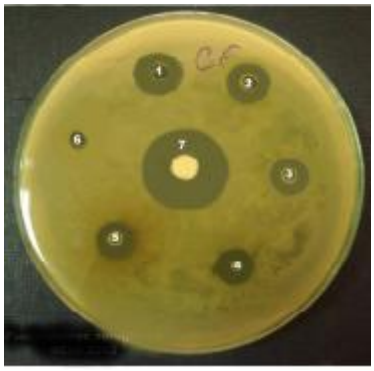
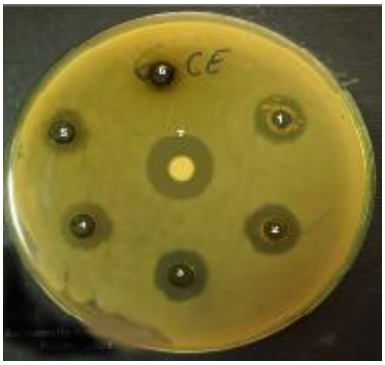
Figure 1: Picture of Embelia ribes.

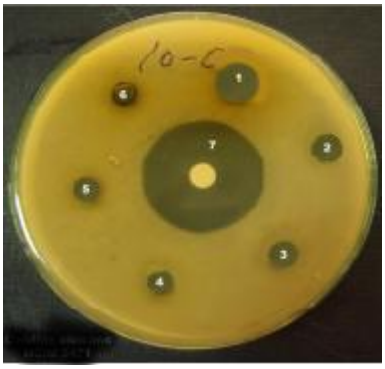

Shigellasonnei


Escherichia Coli


Klebsiella pneumoniae


Staphylococcus aureus


Salmonella typhi


Pseudomonas aeruginosa

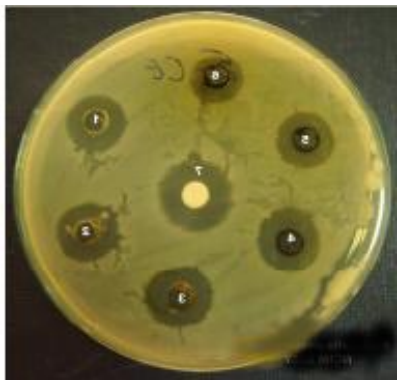

Shigellaflexneri

Figure 2: Antimicrobial activity of ethanolic extract of Embelia ribes whole plant 1.500; 2. 250; 3. 125; 4. 62.5; 5.31.25; 6.15.62 (mg/ml)

Table 1: Antimicrobial activity of ethanolic extract of Embelia ribes.

Sl. No	Organism	Zone of Inhibition (in mm)						Standard
		500 Standard (mg/ml)	250 (mg/ml)	125 (mg/ml)	62.5 (mg/ml)	31.25 (mg/ml)	15.62 mg/ml)	
1	S. aureus	25	15	10	-	-	-	1
2	St. pyogenes	21	13	8	-	-	-	2
3	E. coli	-	-	-	-	-	5	3
4	K. pneumonia	25	23	20	18	13	10	4
5	S. typhi	20	15	10	-	-	-	5
6	S. flexneri	22	19	18	16	-	-	6
7	S. sonnei	25	16	13	10	5	-	7
8	P. mirabilis	12	10	8	5	-	-	8
9	P. aeruginosa	25	16	11	9	-	-	9

Table 2: Physico-chemical parameters of Embelia ribes seeds.

Sl. No	Analytical Parameters	Result
1	Loss on drying at 105°C, % w/w	10.33
2	Total ash, % w/w	12.25
3	Water soluble ash, % w/w	6.63
4	Alkalinity of water soluble ash, ml of 0.1N HCl/g of sample	4.10
5	Acid insoluble ash, % w/w	1.03
6	Water soluble extractives, % w/w	13.25
7	Ethanol soluble extractives, % w/w	6.60
8	Soxhlet Extraction	
i	Hexane, % w/w	1.35
ii	Chloroform, % w/w	1.23
iii	Ethanol, % w/w	8.38
9	pH (10% solution)	7.80