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Phytochemical analysis of *Embelia ribes* seeds for antimicrobial activities

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Abstract
Vayu vilanga a common name of *Embelia ribes*, is a medicinally valuable, woody climber and is well-known drug in Ayurvedic system. In our study efforts were made to prepare seed extracts using solvents with increasing order of their polarity. When an antibacterial assay was carried out the highest activity was recorded by methanolic extract against *P. aerugenosa* while the lowest activity was seen in ethyl acetate extract against *E. coli* and *P. aeruginosa*. In an antifungal assay, the highest activity was observed in methanolic extract against *A. Niger* while the lowest activity was recorded by acetone extract against *A. flavus*. The acetone extract in generally showed lower antimicrobial activity compared to the methanolic extract.

Keywords: *Embelia ribes*, antibacterial activity, antifungal activity phytochemical

1. Introduction
Plants are source of large amount of drugs belonging to different groups such as antispasmodics, emetics, anti-cancer, antimicrobials *etc*. Many plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. The curing ability of plant/extract is attributed to the presence of phytochemical constituents within them. Phytochemicals naturally occur in the leaves, seeds, stem bark, fruits and roots of medicinal plants that have defence mechanism and protect from various diseases. Natural products from plants called secondary metabolites are the end products of primary metabolites which include alkaloids, steroids, flavonoids, terpenoids, glycosides, saponins, tannins, phenolic compounds etc. The active principle of many drugs found in plants is secondary metabolites.
E. ribes is a large woody tropical forest shrub. The root, seeds and leaves of *E. ribes* is used in herbal formulas and formulations. According to Ayurveda, *E. ribes* is cooling tonic, diuretic and laxative. The fruit of *E. ribes* is a good appetizer, carminative, anthelmintic, alexiteric, laxative, alterative, cures tumour, ascites, bronchitis, mental diseases, dyspnoea, and diseases of heart, urinary discharges, jaundice, hemicrania and worms in wounds. The seeds are of high repute as anthelmintic particularly for tapeworms. Seed paste is used against ringworm and other skin diseases. The decoction of dried fruits and seeds of *E. ribes* are frequently for treatment of fevers, paste made out of fruits are applied for efficient wound healing and also for various skin diseases, the fruit of *E. ribes* in powdered form are also taken with milk followed by a purgative is been used to eliminate tapeworm from ancient times [1]. It is one of the ingredients of Koflet cough syrup. In the preparation of Ayurvedic antidiarrhoel drug Diarex for infants and children, dry berries are used.

2. Materials and Methods
2.1 Extraction and qualitative analysis of the secondary metabolites
Collection of seeds
E. ribes seeds were collected from a locally situated medicinal practitioners and Ayurveda outlets.

2.2 Preparation of seed extracts
The dried plant material was subjected to pulverization to get coarse powder and it was extracted by continuous hot percolation method using soxhlet apparatus in various solvents such as Petroleum ether, Ethyl acetate, Acetone and Methanol according to their increasing strength of polarity.

2.3 Antibacterial Sensitive Test: Agar well diffusion method

Four strains of bacteria two gram positive, *Bacillus cereus* and *Staphylococcus aureus* and two gram negative *Escherichia coli* and *Pseudomonas aeruginosa* were selected respectively for the study. The antifungal activity was evaluated against Aspergillus *niger*, *Aspergillus flavus* and *Candida albicans*.

The seeds extract were dissolved in dimethyl sulphoxide (DMSO) at the concentrations of 10mg/mL and further diluted (1:3, 1:1, 3:1) with DMSO and used as stock.

LB agar and potato dextrose agar (PDA) media were prepared, autoclaved at 121 °C for 15 minutes at 15 Lbs, poured in sterile petri plates up to a uniform thickness of approximately 10 - 15 minutes and then the agar was allowed to set at ambient temperature. The wells were made in media after inoculation with the microorganism. 100 µL of inoculums were spread over LB agar plates using sterile spreader, after few minutes wells were made in each petri plated and loaded with 100 µL of extracts, control. Plates were incubated at 37 °C for 24 hrs. Antibacterial activity was observed by measuring its inhibition length. Inhibition length against bacteria was calculated [2, 3]. The experiments were done in triplicate.

Inhibition length = Zone of Inhibition (mm) – Well diameter (mm)

3. Results

3.1 Antibacterial activity

In antibacterial activity, the highest activity (zone of inhibition 0.95 cm) was observed by the methanolic extract of *E. ribes* seeds against *P. aerugenosa* while the lowest activity (diameter of zone of inhibition 0.65 cm) was demonstrated by the ethyl acetate extract against *E. coli* and *P. aeruginosa*. The ethylacetate extract generally showed lower against the test organisms compared to the methanolic extract (Table 1).

Table 1: Zone of Inhibition (cm) in Antibacterial studies

Bacteria	Petroleum ether extract				Ethyl acetate extract					Acetone extract				Methanol extract						Aqueous extract
Dilution	1:3	1:1	3:1	S	1:3	1:1	3:1	S	1:3	1:1	3:1	S	1:3	1:1	3:1	S	1:3	1:1	3:1	S
B. cereus	0.85	0.9	0.8	0.75	0.65	0.75	0.7	0.75	0.7	0.75	0.7	0.75	0.8	0.75	0.75	0.85	0.7	0.8	0.75	0.7
S. aureus	0.8	0.8	0.85	0.8	0.75	0.73	0.8	0.75	0.75	0.85	0.75	0.8	0.7	0.7	0.7	0.7	0.85	0.8	0.8	0.95
E. coli	0.8	0.8	0.85	0.85	0.8	0.7	0.65	0.7	0.75	0.7	0.75	0.7	0.75	0.7	0.7	0.7	0.75	0.8	0.9	0.8
P. aeruginosa	0.7	0.75	0.7	0.8	0.8	0.7	0.65	0.65	0.75	0.7	0.7	0.7	0.75	0.8	0.8	0.95	0.7	0.7	0.7	0.7

(S=stock solution)

3.2 Antifungal activity

In antifungal study the highest activity (zone of inhibition 2.2 cm) was observed by the methanolic extract of *E.ribes* seeds against *A.niger* while the lowest activity (diameter of zone of inhibition 0.5 cm) was demonstrated by the acetone extract against *A. flavus*. The acetone extract generally showed lower against the test organisms compared to the methanolic extract (Table 2).

Table 2: Zone of Inhibition (cm) in Antifungal studies

Bacteria	Petroleum ether extract					Ethyl acetate extract				Acetone extract				Methanol extract					Aqueous extract	
Dilution	1:3	1:1	3:1	S	1:3	1:1	3:1	S	1:3	1:1	3:1	S	1:3	1:1	3:1	S	1:3	1:1	3:1	S
A. niger	1.2	1.1	1.2	1.0	1.2	1.0	1.1	1.2	1.2	1.2	1.1	1.2	2.2	1.2	1.2	1.4	1.2	1.5	1.4	1.2
A. flavus	1.2	1.2	1.1	1.2	1.2	1.2	1.2	1.1	1.3	1.2	0.5	1.3	1.5	1.7	1.4	1.2	1.1	0.9	1.2	1.2
C. albicans	1.0	1.1	1.2	1.2	1.2	1.0	1.2	1.1	1.2	1.2	1.2	1.2	1.0	1.1	1.4	1.5	1.3	1.5	1.8	1.2

(S=stock solution)

4. Discussion

A numbers of medicinal plants that are being used in curing general health of the common people in Ayurvedic and other alternative traditional medicine are not efficient because of several reasons [4]. Different parts of plant consist of different antibacterial activity because of their secondary metabolites that are present over there [2]. Against all the tested bacterial strain, methanol extract of the sample showing much better antibacterial activities in contrast to other extracts, which may be because of organic nature of methanol and also for the reason of its high capacity to dissolve more organic and active antimicrobial compounds [5]. These results substantiated the reports that methanol is a better solvent for more consistent extraction of antimicrobial substances from medical plants compared to other solvents [6-12].

The mechanism of antimicrobial activity by different phytochemicals varies. The mechanism action of alkaloids shows antimicrobial activity by intercalating the DNA or inhibiting the biosynthesis of membrane phospholipids [13]. The terpenoid compounds exhibit antibacterial activity through the mechanism of membrane disruption [14]. Saponins mostly are soap forming compounds that also have antimicrobial property and their action of mechanism is causing the leakage of the protein or certain enzyme from the bacterial cell [15]. Tannins are polyphenol compounds that well known with its protein inhibition property. Tannins interfere the process of protein synthesis by binding to the proline rich protein [16]. Tannins show antimicrobial activities by coagulating the protoplasm of microorganisms [16, 17]. Even though acetone extract contained more phytochemicals, it exhibits lowest antifungal activity. This may be due to unavailability of fungicidal content in small quantity of plant extract.

5. Conclusion

Author concludes from the above investigation that methanolic extract of *E. ribes* seeds can be utilized as an active antimicrobial agent against microbial diseases. Further research is needed toward isolation and identification of active principles present in the extracts which could possibly be exploited for pharmaceutical use.

6. References

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