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EVALUATION OF ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF EPICARP AND ENDOCARP PARTS OF *ELAEOCARPUS GANITRUS*

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ABSTRACT

In this present study, *in vitro* antimicrobial activity of methanol, and acetone extracts of epicarp and endocarp of *Elaeocarpus ganitrus* were investigated. The extracts exhibited antimicrobial activities with zones of inhibition ranging from 9.5mm to 21.0mm and 10.5mm to 22 mm for methanol and acetone extracts respectively. The minimum inhibitory concentration (MIC) of the epicarp acetone extract against different microorganisms was ranged between 0.5 to 0.8 mgml¯1, while that of the endocarp acetone extract ranged between 0.6 to 1.0 mgml¯1. Again all the extracts exhibited appreciable activity against the fungal species were investigated. The zones of inhibition exhibited by the extracts against the test fungal species ranged between 9 to18mm and 10 to 21 mm for methanol and acetonic extracts respectively. Phytochemical screening revealed the presence of tannin, glycosides, alkaloids, quinines, steroid, cumarins, phenols and flavonoids in the extracts. The ability of the crude epicarp and endocarp extracts of *E.ganitrus* to inhibit the growth of bacteria and fungi is an indication for its broad spectrum antimicrobial potential which may be employed in the management of microbial infections.

KEY WORDS: ***Elaeocarpus ganitrus* , antimicrobial activity, phytochemical screening, minimum inhibitory concentration (MIC).**

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INTRODUCTION

Elaeocarpus sp. belongs to the family *Elaeocarpaceae*. This family contains approximately 350 species, which are distributed in India, Southeast Asia, Malaysia, southern China, Japan, Australia, New Zealand, Fiji and Hawaii [1]. It is used in folk medicine in treatment of stress, migraine, anxiety, palpitation, nerve pain, epilepsy, depression, lack of concentration, asthma, hypertension, liver diseases and arthritis. *E.ganitrus* beads are formed of different combinations of carbon, hydrogen, oxygen, nitrogen and different trace elements. According to the ayurvedic medicinal system, wearing of rudraksha can have a positive effect on heart and nerves[2, 3]. The bead is attributed with ability to reduce body temperature and having calming effect[4]. Besides, beads have electromagnetic and electrical properties giving them several healing powers. The important chemical constituents of the leaves, fruits and seeds of *E.ganitrus* account for several medicinal properties of Rudraksha. Rudraksha seeds are dynamically polar and also possess both paramagnetic and diamagnetic properties which facilitate beneficial healing capacity to them. Rudraksha beads confer anti ageing properties depend on their electromagnetism[5]. Seeds are covered by an outer shell of blue color when fully riped, and for this reason they are also known as blue berry beads. The epicarp forms the tough outer skin of the fruit, which bears oil glands and pigments. The epicarp is sometimes called the exocarp. Endocarp (Gr. "inside" + "fruit"), is a botanical term for the inside layer of the pericarp (or fruit), which directly surrounds the seeds[6]. Several studies have confirmed the antimicrobial efficacy of different *Elaeocarpus* species; however, there is insufficient information regarding the antimicrobial activities of *E.ganitrus*. Whatever limited information available on the medicinal properties of *E.ganitrus* is mostly on the leaf extracts of the plant. In this paper, the antimicrobial property of crude extracts of the seed (endocarp and epicarp) of *E.ganitrus* has been studied as part of the exploration for new and novel bio-active compounds.

MATERIALS AND METHODS

Plant materials and preparation of extract

Fresh authenticated *E. ganitrus* Roxb (Rudraksh) seeds were collected from Rudraksha Research and Testing Laboratory (RRTL), Mumbai, and Nagpur City, India. The fresh epicarp and endocarp were air-dried to constant weight and stored in an air-tight container for further use. Exactly 10 g each of the epicarp and endocarp material were cold extracted in methanol and acetone separately by maceration method. Methanol and acetone used were of analytical grade. After 48 hours, the methanolic and acetonic mixtures were filtered through Whatman No.1 filter paper. These extracts were condensed by using a rotatory evaporator.

Test microorganisms

The test microorganisms used in this study (bacteria: *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, and *Micrococcus luteus*; fungi: *Fusarium solani*, *Aspergillus flavus*, *Aspergillus niger* and *Penicillum notatum*) were obtained from the Microbial type culture collections of India. The bacterial isolates were first sub-cultured in a nutrient broth and incubated at 37°C for 18 hours while the fungal isolates were sub-cultured on a Sabouraud dextrose agar (SDA) for 72 hours at 25°C.

Phytochemical analysis of the plant extract

The extracts were subjected to phytochemical tests[7] for plant secondary metabolites, tannins, saponins, steroid, alkaloids, flavonoids, carbohydrates, proteins, quinones, coumarins, phenol and glycosides.

Antibacterial activity

The antibacterial activity of the crude extracts was determined in accordance with the agar-well diffusion method described by Irobi et al.[8]. The bacterial isolates were first grown in a nutrient broth for 18 hours before use and standardized to 0.5 McFarland standards (10^6cfu/ml). Two hundred microliter of the standardized cell suspensions were spread on a Mueller-Hinton agar. Wells were then bored into the agar using a sterile 6 mm diameter cork borer. Approximately 100 µl of the crude extract (10mg/ml) were introduced into the wells, allowed to stand at room temperature for about 2 hours and then incubated at 37°C. Controls were set up in parallel using the solvents that were used to reconstitute the extract. The plates were observed for zones of inhibition after 24 hours. The effects were compared with those of streptomycin and ampicillin at a concentration of 1 mg/ml and 10µg/ml respectively.

Antifungal activity

The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) at 25°C until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. The harvested fungal spores and bacterial isolates were standardized to $O.D_{600nm}$ = 0.1 before use. One hundred microliter of the standardized fungal spore suspension was evenly spread on the SDA using a glass spreader. Wells were then bored into the agar media using a sterile 6 mm cork borer and the wells were filled with the solution of the extract taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour to allow proper diffusion of the extract into the media. Plates were incubated at 25°C for 96 hours and later observed for zones of inhibition. The effect of the extract on fungal isolates was compared with amphothericin B and miconazole at a concentration of 1 mg/ml.

Minimum inhibitory concentration (MIC)

The estimation of MIC of the crude extracts was carried out using the method of Akinpelu and Kolawole[9]. Two-fold dilutions of the crude extract was prepared and 2 ml aliquots of different concentrations of the solution were added to 18 ml of pre-sterilized molten nutrient agar and SDA for bacteria and fungi respectively at 40°C to give final concentration regimes of 0.050 and 10 mg/ml. The medium was then poured into sterile

Petri dishes and allowed to solidify. The surface of the medium was allowed to dry under laminar flow before spreading with 18 hour old bacterial and fungal cultures. The bacterial plates were incubated at 37°C for 24 hours and at 25°C for up to 72 h for bacteria and fungi respectively, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test microorganism.

RESULTS AND DISCUSSION

Antibacterial activity

All four extracts of the epicarp and endocarp tested showed a varying degree of antibacterial activities against the test bacterial species (Table I). The antibacterial activities of the methanol and acetone extracts compared favourably with that of two standard antibiotics (streptomycin and ampicillin) and have appeared to be broad spectrum as its activities were independent on gram reaction. The acetone extract of both epicarp and endocarp (inhibition zone 15 - 22 mm) was found to be more effective than the methanol extract (inhibition zone 11.25 – 21.25 mm) against all test organisms. The minimum inhibitory concentration (MIC) of an acetone extract of epicarp for different organisms ranged between 0.5 and 0.8 mg/ml, while that of the acetone extract of endocarp ranged between 0.6 and 1.0 mg/ml (Table II)

Table I

Antibacterial activities profile of four extracts from the seeds of E.ganitrus.

Test bacteria	Zone of inhibition in mm					
	Methanol		Acetone		ST	AMP
	Epicarp	Endocarp	Epicarp	Endocarp	+ve C	+ve C
Escherichia coli	20.75	12.75	21.5	15.5	0.00	14.75
Staphylococcus aureus	21.0	18.5	20.5	22.0	21.5	0.00
Pseudomonas aeruginosa	15	15.5	18.0	18.5	23.0	0.00
Micrococcus luteus	20.25	13.5	21.75	17.25	15.5	17.25

ST- Streptomycin; AMP-Ampicillin

Table II

The MIC of the extracts of the seeds of E.ganitrus

Test bacteria	Epicarp Acetone (mg/ml)	Endocarp Acetone (mg/ml)
Escherichia coli	0.8	1.0
Staphylococcus aureus	0.5	0.6
Pseudomonas aeruginosa	0.75	0.8
Micrococcus luteus	0.6	0.95

MIC- minimum inhibitory concentration

The inhibitory effect of the extract of *E.ganitrus* against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by these pathogens.

Antifungal activity

The four extracts showed broad antimycotic activity against the tested fungal isolates at a final concentration of 10 mg/ml (Table III) and the performance of the three extracts were similar to the antibacterial activity. The susceptibility of these fungi to *E. ganitrus* extracts is significant, as most of these fungi have recently been implicated in cases of immuno-compromised patients who frequently develop opportunistic infections. Generally the acetonic extract had the highest activity against both bacterial and fungal isolates. This was followed by the methanolic extract. The ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad spectrum anti-microbial potential of *E. ganitrus* that makes the plant a better candidate of bioprospecting for antibiotic and antifungal drugs.

Table III

Antifungal activity profile of four extracts from the epicarp and endocarp of E. ganitrus.

Test Fungi	Zone of inhibition in mm				
	Methanol		Acetone		Amphotericin B
	Epicarp	Endocarp	Epicarp	Endocarp	+ve C +ve C
Fusarium solani	10.0	12.5	10.5	11.5	20.25
Aspergillus flavus	13.5	9.5	15.0	10.75	25.0
Aspergillus niger	12.0	10.5	16.5	10.5	24.0
Penicillum notatum	12.5	15.0	22.0	18.5	26.5

Phytochemical screening

Investigations on the phytochemical screening of *E. ganitrus* seed extracts revealed the presence of steroids, tannins, glycosides, alkaloids, quinones, coumarins, phenols and flavonoids (Table IV). These compounds are known to be biologically active and therefore aid the antimicrobial activities of *E. ganitrus*. These secondary metabolites exert antimicrobial

activity through different mechanisms. Tannins have been found to form irreversible complexes with proline rich protein[10] resulting in the inhibition of cell protein synthesis. Parekh and Chanda[11] reported that tannins are known to react with proteins to provide the typical tanning effect which is an important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery[12]. These observations therefore support the use of *E.ganitrus* in herbal cure remedies. Li and Wang[13] reviewed the bio-logical activities of tannins and observed that tannins have anticancer activity and can be used in cancer prevention, thus suggesting that *E.ganitrus* has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in *E. ganitrus* supports the traditional medicinal use of this plant in the treatment of different ailments.

Table IV

Phytochemical constituent of epicarp and endocarp extract of E. ganitrus.

Sl.no	phyto-constituents	Endocarp		Epicarp	
		methanol	acetone	methanol	Acetone
1	Alkaloid	+ve	+Ve	+Ve	+Ve
2	Flavanoid	-Ve	-Ve	+Ve	-Ve
3	Carbohydrates	-Ve	-Ve	-Ve	-Ve
4	Tannins	+Ve	+Ve	+Ve	+Ve
5	Saponins	-Ve	-Ve	-Ve	-Ve
6	Glycosides	+Ve	-Ve	-Ve	-Ve
7	Quinines	+Ve	+Ve	+Ve	+Ve
8	Anthroquinone	-Ve	-Ve	+Ve	-Ve
9	Steroid	-ve	+ve	+ve	+ve
10	Coumarins	+ve	+ve	+ve	+ve
11	amino acid	-ve	-ve	-ve	-ve
12	Protein	+ve	-ve	-ve	-ve
13	Gum	-ve	-ve	-ve	-ve
14	Phenol	+ve	+ve	+ve	+ve

+ve; Presence of compound, -ve; absence of compound

Another secondary metabolite compound observed in the seed extract of *E. ganitrus* was alkaloid. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the elimination and reduction of human cancer cell lines[14]. Alkaloids which are one of the largest groups of phytochemicals in plants have amazing effects on humans and this has led to the development of powerful pain killer medications[15]. Steroidal compounds present in *E.ganitrus* extracts are of importance and interest due to their relationship with various anabolic hormones including sex hormones[16]. Quinlan et al.[17] worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. Neumann et al.[18] also confirmed the antiviral property of steroids. Flavonoids, another constituent of *E.ganitrus* seed extracts exhibited a wide range of biological activities like antimicrobial, anti-inflammatory, anti-angionic, analgesic, anti-allergic, cytostatic and antioxidant properties[19]. Previous studies shows that *E. ganitrus* possesses sedative, hypnotic, tranquillizing, anticonvulsive, antiepileptic and antihypertensive activities. *E.ganitrus* contains quercetin, gallic and ellagic acids, (-) elaeocarpine, (-) iso-elaeocarpine and rudrakine. Hence, the presence of these compounds in *E. ganitrus* corroborates the antimicrobial activities observed.

CONCLUSION

It is concluded that specific solvent shall be used to extract active phytochemicals from epicarp and endocarp of *E. ganitrus, which* could be a potential source of active phyto-constituents such as alkaloids, phenols, flavonoids, tannin, glycosides and coumarins. This above study has confirmed that *E. ganitrus* seeds have antimicrobial activities and could play a major role in pharmaceutical industries, and a detailed assessment of its *in vivo* potencies and toxicological profile is ongoing.

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