Antimicrobial and hypoglycemic effects of *Elaeocarpus Ganitrus* Seed

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ABSTRACT

The Evaluation of the antibacterial, antifungal and hypoglycemic effects of methanol and water (ratio70:30) extract of *Elaeocarpus Ganitrus seeds* were investigated by using the cup bore agar diffusion method and hypoglycemic effect was evaluated in alloxan induced diabetic rats. The extract of *E.Ganitrus seed* significantly inhibited *Bacillus subtilis, Staphylococcus aureus* (Gram positive), *Klebsiella pneumonia, Escherichia coli, Salmonella typhi, Proteus vulgaris* and *Pseudomonas aeruginosa* (Gram-negative) to varying degrees, but it had little inhibitory effect on fungal species (*Aspergillus niger, Aspergillus fumigatus*, *Mucor pusillus* (fungi), and *Candida albicans* (yeast).For anti bacterial study Gentamycin, the standard antibacterial drug used for inhibiting these bacteria. The effect on *B. subtilis and E. coli* were similar to that of gentamycin. Ketoconazole, the regular ant fungicide used was effective against all the fungi and yeast species. The extract of *E.Ganitrus seed* also significantly ($P<0.05$) reduced blood glucose level in alloxan induced diabetic rats twelve hours after administration. The effect of the extract at 1000mg/kg was comparable to that of the standard drug (chlorpropamide) used. Results support some of the traditional uses of *E.Ganitrus seed* as an anti-infective herb and for the management of diabetic sores

Keywords: Antibacterial, Antifungal, Hypoglycemic, *Elaeocarpus Ganitrus.*

INTRODUCTION

Elaeocarpus is a genus belonging to family Elaeocarpaceae contains tropical and subtropical evergreen trees and shrubs. Nearly 360 species of Elaeocarpus trees are found in different parts of the world. Out of these Elaeocarpus species *Elaeocarpus Ganitrus* plant is traditionally use

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vastly for various diseases[1]. As per Ayurvedic system of medicine, wearing *Rudraksha* beads relieves strain, insomnia, anxiety, lack of concentration, depression, palpitation, hypertension, rheumatism, infertility and asthma. It has anti-aging effect also [2]. Ethanol extract of the fruit of *E. ganitrus* have been reported to exhibit sedative, hypnotic, tranquillizing, anticonvulsive, antiepileptic and antihypertensive properties[3,4,5]. It is also found that chitosan Based leaf extract of *Elaeocarpus ganitrus* produced hypoglycaemic effect in normal rats. The chitosan based extract improved the antidiabetic activity of *Elaeocarpus ganitrus* clearly indicating synergism [6]. In vivo antimicrobial activity of methanol and acetone extracts of epicarp and endocarp of Elaeocarpus ganitrus bead were when investigated, the extracts show 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 in 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 when all the extracts exhibited appreciable activity against the fungal species The zones of inhibition exhibited by the extracts next to the test fungal species ranged between 9 to18mm and 10 to 21 mm for methanol and acetonic extracts respectively[7,8]. The present study was consequently designed to find out the antimicrobial and hypoglycemic effects of *E. ganitrus* seeds

MATERIALS AND METHODS

Collection of plant materials: Genuine Elaeocarpus Ganitrus bead Rudrakasha were collected from online seller through EBay India in 2016 and further authenticated by X-Ray, water dipping technique in Maharana Pratap College of Pharmacy Lab .

Extraction of plant materials: The seeds were air dried below shade and then cruse and powdered using surface sterilized mortar and pestle. Powder plant material (1000 grams) was macerated in 70% methanol and left in air tight aspirator bottle for 72 hrs. The extract was filtered subsequent to 72 hrs, with the aid of sterile sieving fabric. The filtrate was then evaporated using a rotary evaporator at 45^0C. A yield of 21% (w/w) was obtained from the extraction process. The dried extract was labeled and stored in an air-tight container for advance use.

Bacteria and fungi species: *Bacillus Subtilis, Staphylococcus aureus* (Gram positive), *Klebsiella pneumonia, Escherichia coli, Salmonella typhi, Proteus vulgaris*, *Pseudomonas aeruginosa* (Gram-negative), *Aspergillus niger, Aspergillus fumigates*, *Mucor pusillus* (fungi), and *Candida albicas* (yeast) were used for this study. The test microorganisms were obtained from the stock cultures of microbiology unit of the Department of Pharmaceutical science, Kanpur, UP, India purchased from Carolina Biological Supply Company Amazon online.

Preparation of test organisms: An inoculums of amount 10^8 colony forming units per milliliter (cfu/ml) of each of the isolates was arranged, this was affected by suspending lapfuls of inoculums from the stock into different labeled test tubes, each containing 10 ml of nutrient broth. A whole of 3 test tubes were used for each test organism. The treated tubes were incubated at 37^0C for 24 hrs. The resultant cultures were then diluted with fresh nutrient broth in order to achieve optical densities corresponding to 10^8 cfu/ml [9, 10].

Antimicrobial susceptibility test: Various dilutions (100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml) of the plant seed extracts of *E. ganitrus* were assayed for antimicrobial activity on the test organisms using modified cup plate method [11]. The Nutrient agar plates and Czapek-dox agar plates were prepared. Loopful (0.002 ml) of each bacterium inoculums corresponding to $1x10^8$ cfu were evenly marked on the prepared agar plate containing Czapek-dox agar**[12].** The plates were then air dried for a period of five minutes. Prepared fungi spore suspension was evenly marked on the prepared agar plate containing potato/glucose fusion **[13].** The plates were then air dried. Cups were bored in each solid media using sterile cork borer (number 3). A volume of 0.1 ml of the prepared dilutions of the extract (100 mg/ml, 75 mg/ml, 50 mg/ml, 25 mg/ml and 12.5 mg/ml, respectively) was given into each of the aseptically bored holes. The plates were replicated 5 times then incubated at 37^0C for one day for bacteria and *C. albicans* and for a period of 5 days for the test fungi. The diameters of the zones of inhibition were calculated and recorded.

Anti-diabetic effect of E.Ganitrus: Wister albino rats (weight 150-250g) were injected with aloxan (100 mg/kg) through tail vein. Five days later, blood glucose levels of the animals were determined using a glucometer. The diabetic rats showing blood glucose levels in the range of 240–400 mg/dl were selected for the evaluation of *E. ganitrus* for antidiabetic properties [14, 15]. The diabetic rats were divided into four groups of six in each. The control group was given 0.1 ml of distilled water, i.p. The test groups were given the extract of *E. ganitrus* (500 mg/Kg and 1000 mg/Kg respectively).

The 4^{th} group received chlorpropamide (400 mg/kg, p.o.). Blood glucose levels were determined at 0, 1, 2, 3 and 12 hours. The rat`s blood was collected from its tail by massaging the whole length of the tail awaiting adequate blood accumulates at the tip[16-17]. The glucometer was switched on and the glucometer code number was set to the code on the one touch glucose strip bottle. Then the strip was inserted into the glucometer as per instruction. With the aid of the surgical knife, the tip of the rat's tail was cut off and the blood dropped on the appropriate portion of the glucose strip inserted in the glucometer. After some seconds, the glucometer starts counting down from 45 seconds to 1 second and then displayed on its screen the glucose concentration in mg/dl. This procedure was repeated for all the rats used in the experiment and their blood glucose level noted.

RESULTS

Anti-microbial activities: The crude extract of *E. ganitrus* subdued ($P<0.05$) the tested bacterial strains (Table 1). The crude extract showed some action against *B. subtilis, E. coli, P. aeruginosa, and P. mirabilis*, but showed marginal activity against *S. aureus K. pneumonia, and S. typhi.* The activity of the extract against *B. subtilis and E. coli* was comparable to that gentamycin (Table 1).

Table-1: Antibacterial effects of methanol/water extract of *E. ganitrus* seeds.

Organisms	Zones of Inhibition (mm) Extract Concentration					
	100mg	**75mg**	**50mg**	**25mg**	**12.5mg**	**Gent. (4mg)**
E. coli	30.45±4.6*	26.02±2.7*	23.48±3.1*	19.2±0.5*	19.3±0.2	21.2±0.4
B. subtilis	22.15±3.43*	21.21±2.9*	15.9±2.56	10.7±0.4*	12.3±0.5	16.5±2.8
P. mirabilis	19.05 ±0.7	18.1 ± 2.9*	13.4 ± 1.1*	8.1 ± 1.8*	0.5±0.2*	22.8±1.4
P. aeruginosa	5.7±1.1*	5.3 ± 1.9*	5.13 ± 2.1*	4.4 ± 0.4*	0.9±0.6*	13.0±1.7
S. aureus	2.0 ±1.5*	2.6 ± 0.6*	1.7 ± 0.5*	1.3 ± 0.5*	1.0±0.8*	24.0±1.9
S. typhi	1.9±0.7*	0.7±0.5*	0.0±0.7*	0.0±0.9*	0.0±0.0*	21.8±0.4
K. pneumonia	1.7±0.7*	1.3±0.6*	1.9±0.5*	0.8±0.7*	1.3±0.6 *	19.0±0.5

- Results were expressed as Mean ± SEM.
- *P<0.05. When compared with gentamycin.
- Gent.= Gentamycin

The crude extract of *E. ganitrus* mildly inhibit (P>0.05) the tested fungi and yeast strains (Table 2). The crude extract showed very little activity against *A. fumigatus A. niger C. albicans and M. pusillus.* Ketoconazole was very effective in inhibiting all the fungi used for this study (Table 2).

Table-2: Antifungal effects of methanol/water extract *E. ganitrus* seeds of plant.

Organisms	Zones of Inhibition (mm) Extract Concentration					
	100mg	**75mg**	**50mg**	**25mg**	**12.5mg**	**Keto.** (1mg)
A fumigatus	0.7±0.5*	0.6±0.3*	0.4±0.2*	0.4±0.3*	0.3±0.1*	16±5.4
A. niger	0.5±0.1*	0.9±0.1*	0.5 ± 0.2*	0 ± 1*	0 ± 3*	13±1.5
C. albicans	0 .2± 0.2*	0.2 ± 0.1*	0 ± 0*	0 ± 3*	0.3 ± 0.1*	23±2.8
M. pusillus	0 ± 3*	0 ± 1*	0 ± 0*	0 ± 2*	0 ± 0*	25±2.1

- Results were expressed as Mean ± SEM.
- *P<0.05. When compared with ketoconazole.
- Keto. = Ketoconazole

E. ganitrus crude extract has great potential as antimicrobial agent against bacteria but not so much effective against fungi and it can be used in the treatment of infectious diseases caused by resistant bacteria. *E. coli* showed maximum susceptibility

Anti-diabetic effect: *E. ganitrus* crude extract significantly (P<0.05) reduced blood glucose level in aloxan induced hyperglycemic rats. The effect of *E. ganitrus* at 1000 mg/Kg was comparable to that of chlorpropamide (Table 3). The hypoglycemic effect of *E. ganitrus* was not evident until the 12th hour of administration; this implies that its hypoglycemic effect is not immediate.

Table-3: Effect of extract *E. ganitrus* on BGC Level of hyperglycemic rats.

Treatment (mg/kg)	**Mean BGC level ±S.E.M. (mg/dl) Sampling time (hours)**				
	0	**1**	**2**	**3**	**12**
Control	248.57±92.85	235.66±34.45	226.16±16.78	265.32±20.76	280.49±21.83
Chlorpropamide, 400mg/Kg	297.27±120.54	306.41±98.47	326.65±109.72	267.45±78.68	52.88±10.07*
M. balsamina 500mg/Kg	248.42±70.62	269.83±61.79	304.40±97.93	211.29±18.89	67.22±6.18*
M. balsamina 1000mg/Kg	355.77±73.83	416.55±155.07	356.33±66.38	345.65±101.42	34.25±4.11*

- *P < 0.05, there is significant difference.
- The values were expressed as mean ±S.E.M.
- BGC= Blood glucose concentration.

DISCUSSION

Since the ancient time mankind have been striving hard for sound health and peace of mind, but due to modern life style polluted fruits and vegetables, stress and anxiety, lack of physical exercise and environment pollution we are far from achieving our ultimate goal of sound health. Despite such type of health problems Mother Nature has the potential to provide us all type of foolproof solutions for our health problems. The nature remains as the potential source of organic structures of unparalleled diversity. Over the earlier decades there has been a lot of effort in the area of elucidating the lively principle of herbal medicines and synthesizing the active ingredient for medical use. Research has exposed that a number of powerful herbs don`t show activity or show reduced activity after separation and synthesis of the active principle. This largely has been credited to the fact that a number of of the components in the plants acts synergistically or diminish

the proceedings of other components in the plant. The use of the plant stuff has been supported by this detection. The crude extract of the seeds of *E. ganitrus* showed antibacterial and antidiabetic effects.The methanolic extracts of beads of the plant *E. ganitrus* was active against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Salmonella typhi* and not effective against *Saccharomyces cerevisiae* and *Aspergillus niger*. This work also established that the plant has activity against *B. subtilis, P. mirabilis and K. pneumonia,*. This suggests that *E. ganitrus* has a broad spectrum anti bacterial activity against Gram negative and Gram positive bacteria. The crude extract showed very little activity against *A. fumigatus A. niger C. albicans and M. pusillus so* it is not so much effective against fungi. Above experiment implies that the *E. ganitrus* alone will effectively combat the infection and pain associated with infectious and diabetic diseases thus reducing the number of drugs used in pain, infection and diabetes administration and preventing the complications of poly pharmacy. *E Ganitrus* methanolic crude extract reduced blood glucose level in aloxan induced hyperglycemic rats. The hypoglycemic effect of *E. ganitrus* was not evident until the 12^{th} hour of administration so clearly implies that its hypoglycemic effect is not immediate.

CONCLUSION

E. ganitrus crude extracts have great potential as antimicrobial agent against bacteria but not against fungi and it can be used in the treatment of infectious diseases caused by resistant bacteria strains. The antibacterial and hypoglycemic properties of *E. ganitrus* shown in this study support the traditional use of the plant in the management of diabetic sore.

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