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Original Article

PHYTOCHEMICAL EVALUATION AND ANTIHYPERGLYCEMIC EFFECTS OF *ELAEOCARPUS GANITRUS* ROXB (*RUDRAKSHA*) IN STREPTOZOTOCIN INDUCED DIABETES

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ABSTRACT

Objective: Present study was aimed at examining the constituents of essential oil of leaves and evaluation of phytochemical and antihyperglycemic effect of methanolic seed extracts of *Elaeocarpus ganitrus* Roxb. in Streptozotocin induced diabetes.

Methods: Essential oil was extracted from *E. ganitrus* leaves by hydrodistillation and purified oil was subjected to GC-FID analysis Preliminary phytochemical screening of various extracts of *E. ganitrus* seeds was carried out and antidiabetic activity of methanolic extract was evaluated in streptozotocin induced diabetic rats.

Results: The results revealed which showed the presence of altogether sixteen constituents. The methanolic seed extract of *E. ganitrus* exhibit potent antidiabetic activity comparable to the standard drug glybenclamide.

Conclusion: It can be concluded from the study that methanolic extract of *E. ganitrus* seeds possess potent hypoglycemic activity.

Keyword: Antihyperglycemic, Antidiabetic, Hypoglycemic

INTRODUCTION

India, with its diverse agro-climatic conditions and regional topography, has been considered as the treasure house or botanical garden of plant genetic resources. India is also recognized as one of the world's top 12 mega diversity nations. It has been widely observed in developing countries that, the use of traditional medicines are common to the maintenance of the health [1]. In the developing countries, for the treatment of minor ailments, and cost for personal health maintenance, herbal medicines have become more popular [2]**.** The genus *Elaeocarpus* includes approximately 350 species which are widely distributed from Madagascar in the west to New Zealand in the east comes under subtropical evergreen trees and shrubs [3]. *Elaeocarpus ganitrus* Roxb. also known as *Elaeocarpus sphaericus* Geertn. K. Schum. is commonly known as Rudraksha (in Hindi) or Bead Tree (in English). The tree occupies areas starting from Manila, Philippines through Myanmar to whole Northeast India, Bangladesh, Nepal and Bhutan [4]. In India the tree occupies the regions ranging from the Gangetic plains to the foothills of the great Himalaya. Despite being a sacred tree of great mythological and medicinal importance, Rudraksha has traditionally been used for the treatment of various ailments like stress, anxiety, depression, nerve pain, epilepsy, migraine and lack of concentration etc. Regardless of important progress in the management of diabetes using synthetic drugs, many traditional plants treatments are still used throughout the world. However, few traditional plants have received proper scientific intervention. Considering its immense therapeutic significance and extensive use of *E. ganitrus* in Ayurvedic medicine for centuries, it was thought imperative to evaluate the species phytochemically and pharmacologically in support of its therapeutic use [5].

Therefore, the present study was intended to undertake phytochemical evaluation of leaves and seeds of *E. ganitrus* and to evaluate the hypoglycemic effect of seed extract in streptozotocin induced diabetic rats and compare its efficacy with the standard oral hypoglycemic drug Glibenclamide. Extensive literature revealed that the Gas chromatography of the essential oil from the *E. ganitrus* leaves has not been reported previously and there are limited reports available regarding the antidiabetic activity of the seed.

MATERIAL AND METHODS

Plant materials

Seeds and leaves of *E. ganitrus* were collected from the Central Nursery of Forest Research Institute (FRI), Dehradun, Uttarakhand, India and were authenticated by Botany Division, FRI. A voucher specimen was preserved in the Chemistry Division, FRI for future reference. Cleaned and shade dried seeds were ground to coarse powder using an electric grinder for further studies while fresh leaves were used for the extraction of essential oil.

Chemical reagents

All the chemicals and reagents used for the analytical works were of laboratory grade and refer to SD Finechem, Ranbaxy. TLC plates were prepared with silica gel G (Qualigens).

Determination of extractive values

E. ganitrus seeds were extracted exhaustively and sequentially using petroleum ether, acetone and methanol in the order of increasing polarity and corresponding extractive values were determined following standard protocol [6-10].

Phytochemical screening

Preliminary phytochemical screening of petroleum ether, acetone and methanol extracts of *E. ganitrus* seeds was performed to detect the presence and/or absence of various phytoconstituents like alkaloids, carbohydrates, glycosides, sterol, phenolics, flavonoids and saponins following standard methods [7-8].

Estimation of phytoconstituents by chromatographic techniques

TLC profiling

The clean glass plates were coated with the slurry of TLC grade silica gel G by spreading method, then dried and activated. TLC plates spotted with extracts were developed using various solvent systems so as to attain finer resolution of spots. Visualization of compounds on developed plates was normally carried out by 5% sulphuric acid or by other suitable detecting agents followed by heating at 100°C until colour appears [11-18].

Essential oil extraction

Essential oils were isolated from fresh leaves of *E. ganitrus* Roxb. by hydro distillation method using a Clevenger type apparatus for 6 hrs. The oil was collected, measured and separation of oil from the aqueous part was done by dichloromethane with the help of a separating funnel, dehydrated over anhydrous sodium sulphate and filtered, the filtrate was subjected to rotary evaporator to separate

the oil from dichloromethane and the oil was stored in amber vials at cool and dark place prior to analysis.

Analysis of the essential oils

GC-FID analysis of the oil was carried out on a chemito gas chromatograph (Chemito) fitted with a column (30m, 0.25 mm, id-BP5, 0.25 µm). Oven temperature 60°C (2 min) then 5°C increment goes to 300°C (10 min) carrier gas nitrogen at flow rate 2 ml/min.

Antihyperglycemic activity

Animals

Female Swiss albino mice between 20-25 g were obtained from the animal house and used for toxicity study and male Albino wistar rats between 175-225 g were used for antidiabetic activity. The animals were housed in groups of 6 in polypropylene cages in an animal room with constant temperature (21°C) and 12 h light: dark cycle, with free access to food and water. All procedures were approved by the institutional animal experimentation ethics committee and in line with the NIH guideline for the use of laboratory animals.

Acute toxicity study

The male Swiss albino mice were fasted for 3 h prior to the experiment. Acute toxicity study (ATS) was being carried out by injecting 4 doses (100 mg, 200 mg, 500 mg and 1.00 g/kg, p. o) of the methanolic extract to different mice, groups (n = 3). The mortality and general behaviour of the mice was observed for 48 h [19] with special attention to the first 30 min and the first 4 h after the single oral administration, then periodically during the 48 h and daily for a total of 2 weeks [20, 21].

Induction of diabetes

The rats were fasted overnight before the administration of Streptozotocin. Diabetes was induced in rats by intraperitonial injection of streptozotocin dissolved in 0.1 M sodium citrate buffer pH 4.5 at the dose of 50mg/kg body weight. The animals were allowed to drink 5% glucose solution overnight to overcome hypoglycemic shock. The development of diabetes was confirmed after 48 h of streptoozotocin injection. The animals having fasting blood glucose level more than 200 mg/dl were considered as diabetic rats and used for the experiment [22, 23], diabetic animals were grouped five days after induction of diabetes to study the effect of methanolic extract of *E. ganitrus* seeds in streptozotocin induced diabetic rats.

Experimental design

Six groups of normal and diabetic rats (n=5) were used. The control and diabetic groups received 1.5% dimethylsulphoxide in distilled water. Four groups each of normal and diabetic rats were given 40, 75, 150 and 300 mg/kg p. o. of the methanolic extract of *E. ganitrus* seeds respectively. Their positive control was treated with 5 mg/kg glibenclamide. Blood glucose level was determined at zero and subsequently at time 0.5, 1, 2, 3, 5 and 8 h.

Estimation of blood and urine glucose

Blood samples (20 µl) we obtained from tail tip of fasted rats and the blood glucose level was determined using a glucometer (Accutrend GC, Boerhinger, Mannheim Germany) while the glucose in fresh urine was assessed using glucose indicator sticks before and after treatment.

Statistical analysis

All the values in the test are presented as mean ± SEM. Statistical differences between the means of various groups were statically analyzed by one way analysis of variance (ANOVA) using SPSS program followed by students T-test. P values of <0.05 or less were considered to be significant.

RESULTS AND DISCUSSION

Extractive value

Extractive values of *E. ganitrus* seeds determined under both exhaustive and sequential extraction are presented in table 1 that provided idea about extractability of seed chemical constituents in different organic solvents.

Plants possess different solubility behaviour in various solvents. The yields of extracts from a plant in different solvents provide information about the solubility of plant chemical constituents in different organic solvents thus suggesting the best solvent for extraction of phytochemicals. It is clear from the table-1 that methanol yielded the highest amount of phyto-constituents in both exhaustive and sequential extraction.

Preliminary phytochemical screening

E. ganitrus seed extracts obtained by exhaustive as well as sequential extraction were subjected to preliminary phytochemical screening and the results are given in table 2.

The qualitative evaluation of exhaustive and sequential extracts of different polar and non-polar solvents revealed the presence of highest amount of phytoconstituents in methanol extracts. Both exhaustive and sequential methanol extracts clearly indicate the presence of alkaloids, carbohydrates, sterols, flavonoids. The presence of a number of secondary metabolites indicated towards the occurrence of significant therapeutic activity.

TLC profiling of seed extracts

Thin layer chromatographic profiles were developed for petroleum ether, acetone and methanol extracts obtained through sequential extraction of *E. ganitrus* seeds. All the three extracts precisely resolved into four spots with solvent system benzene: chloroform: methanol (6:4:1) indicating it to be the appropriate solvent system for TLC profiling of extracts of *E. ganitrus* seeds. The R_f values of all the spots detected were calculated as 0.63, 0.68, 0.74, 0.79, for petroleum ether extract; 0.51, 0.57, 0.61, 0.64 for acetone extract and 0.37, 0.41, 0.54, 0.59 for methanol extract. TLC studies thus made out the most favourable solvent system and corresponding TLC patterns for phytochemical profiling of the *E. granitrus* seed extracts.

GC FID Analysis of leaf essential oil

E. ganitrus leaf oil was subjected to essential oil analysis by GC-FID and the chromatogram is shown in fig. 1**.** The GC analysis showed the presence of 16 major peaks in the chromatogram however no related study was found related to the oil of *E. ganitrus* and due to lack of standards, it was very difficult to identify the compounds that correspond to the peak. The first peak was found to be dominated with a maximum area and height (%), followed by peak no 8 and peak no 2 with 9.21 and 8.09 % area.

Table 1: Extractive values of *E. ganitrus* seeds

Extraction Mode	Extractive Values (w/w)
Exhaustive Extraction (EE)	
Petroleum ether	0.275
Acetone	0.386
Methanol	0.459
Sequential Extraction (SE)	
Petroleum ether	0.0795
Acetone	0.1885
Methanol	0.1902

Table 2: Preliminary phytochemical evaluation of different extracts of *E. ganitrus*

Phytoconstituents	Pet. Ether extract		Acetone extract		Methanol Extract	
	EE	SE	EE	SE	EE	SE
Alkaloids	+	+	+++	+++	+++	+++
Carbohydrates	-	-	-	-	+	+
Glycosides	-	-	+	+	+	+
Sterols	++++	++++	+++	+++	+++	+++
Phenolics	++++	++++	+++	+++	+++	+++
Flavonoids	-	-	-	-	+	+
Saponin	-	-	-	-	-	-

Table 3: GC-FID analysis of essential oil

Peak No.	Retention time	Area %	Height %
1	5.080	30.6705	22.7345
2	5.480	8.0912	8.9021
3	8.113	6.2446	5.1902
4	9.167	3.1353	3.9733
5	11.253	6.1164	5.1182
6	12.127	2.5258	3.8676
7	15.127	4.9408	3.7623
8	17.653	9.2195	11.8108
9	18.460	6.4022	5.4282
10	21.147	3.6531	3.7581
11	22.227	2.7297	2.8794
12	24.147	3.0376	3.8956
13	27.760	1.9402	2.2376
14	31.360	1.3278	2.5048
15	33.487	1.0438	1.9487
16	34.093	3.7230	5.8595

Table 4: Effect of *E. ganitrus* extract in blood glucose and urine glucose level

	Dose (mg/kg)	Blood glucose (mg/dL)			Urine glucose (mg/dL)		
		Before treatment	After treatment	% reduction	Before treatment	After treatment	% reduction
Normal	-	99.0±1.3	98.8±1.9	-0.2	0	0	0
Diabetic control	-	343.0±13.6	347.4±11.2	1.3	4.8±0.2	4.8±0.4	0.02
Extract	40	330.0±39.6	132.6±18.6**	-59.8	4.6±0.2	0.4±0.2**	-91.3
Extract	75	339.2±14.4	192.2±12.3**	-43.3	4.4±0.2	1.6±0.2**	-63.6
Glibenclamide	2IU	379.2±24.2	277.4±35.2	-26.9	4.8±0.2	2.6±0.2**	-45.8

Values are expressed as Mean±SEM, *P<0.05, **P<0.001% reduction of blood and urine glucose

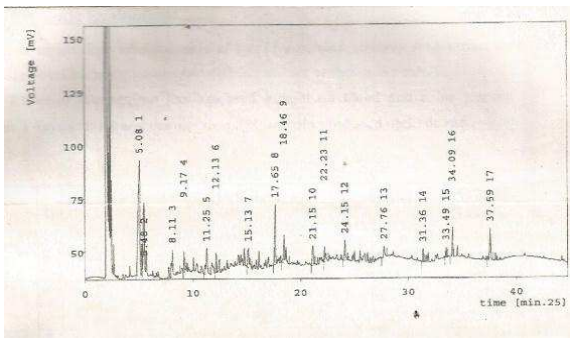

Fig. 1: GC chromatogram of *E. ganitrus* leaf essential oil

Antihyperglycemic activity

Acute oral toxicity

Acute oral toxicity showed that the methanolic extract of *E. ganitrus* seeds was non toxic and caused no mortality upto 1 g/kg. However it was also observed previously that the LD_{50} value of *E. ganitrus* was more than 5g/kg [24].

Effect of *E. ganitrus* extract in normal rats

The hypoglycemic effect of the methanolic extract were significant (P<0.05) after one hour of dosing at 40, 75 and 150 mg/kg, maximum reduction was observed at 40mg/kg body weight.

Effect of *E. ganitrus* extract in diabetic rats

The dose 40 mg/kg and 75 mg/kg were found significant in the diabetic rats. Positive control Glibenclamide had its significant effect 3 h post dosing, whereas methanolic extract showed significant effect 5 h post dosing. Twice daily administration of the extract for three days decreased the blood and urine glucose levels in diabetic rats (table 3). 40 mg/kg and 75 mg/kg of the extract significantly (P<0.05) reduced blood glucose level by 60% (P<0.001) and 42.8% respectively compared to 27% produced by insulin (P<0.05).

The results confirmed the corresponding reduction (**P<0.001) of urine glucose levels by 91.0%, 64.0%, at 40 and 75 mg/kg of the methanolic extract and 46.0% for insulin whereas no significant change was observed in body weight, food and water intakes of animals. Streptozotocin induced hyperglycemic effect has described as a useful experimental model to study the activity of hypoglycemic agents [25], streptozotocin selectively destroys the pancreatic insulin secreting β-cells, leaving less active cells and resulting in a diabetic state [25, 26]. Glibenclamide treatment (5mg/kg) was not effective in reducing blood glucose in STZ diabetic rats and normoglycemic rats. It has been reported that glibenclamide was not effective when destruction of β-cells has occurred and hence more effective moderate diabetic rats than in severe diabetic animals [27-29].

The report suggested that a significant (*P<0.05) decrease in the blood glucose level and urine glucose level was observed at a dose of 40 mg and 75 mg/kg body weight (fig. 2, 3) of the methanolic extract.

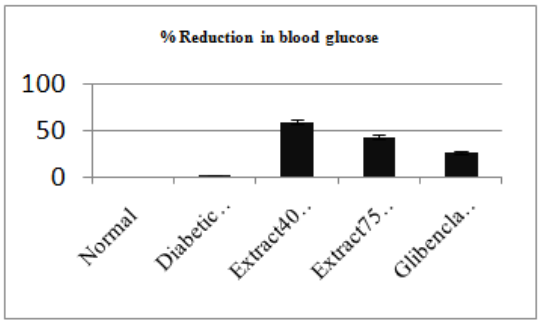

Fig. 2: Effect of *E. ganitrus* seed extract on blood glucose level after 3 days

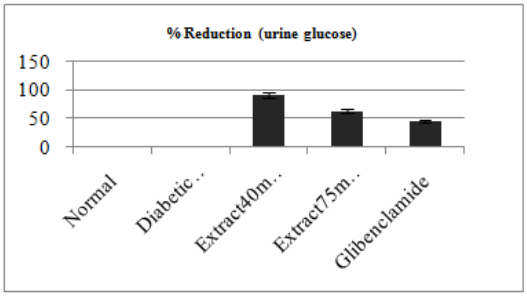

Fig. 3: Effect of *ganitrus* seed extract on urine glucose level after 3 days

The results of the study supported the previous work [24] which showed that seeds of *E. ganitrus* showed antidiabetic activity at 500 mg and 1000 mg/kg. The *E. ganitrus* seeds showed significant anti diabetic potential and further studies are required for the evaluation of active principle responsible for the antidiabetic activity. The Rudraksha tree is well recognize in the religious and spiritual purposes, the beads of Rudraksha is wear in Hindu mythology, it is not just because of its spiritual benefits but also for the medicinal purposes. It is believed that the beads are having electromagnetic properties which regulates the body potential thus to wear the bead near heart is said to be beneficial for various heart related disorders.

CONCLUSION

It is concluded from the study that the *E. ganitrus* seeds have antihyperglycemic activity that could play an important role to reduce the blood glucose levels in diabetic conditions. Moreover, further study on biochemical and pharmacological aspects may elucidate the mechanism of its antidiabetic action. Also, isolation, purification and characterization of bioactive active components from the seeds could be interesting that may pave an exclusive or complementary regiment for the treatment of diabetes mellitus.

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CONFLICT OF INTERESTS

Declared None

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