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SCREENING OF PHYTOCHEMICALS, ANTIOXIDANT AND INHIBITORY EFFECT ON ALPHA-AMYLASE BY ETHANOLIC EXTRACT OF *ELAEOCARPUS GANITRUS* (BARK)

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ABSTRACT: *Elaeocarpus ganitrus* Roxb is widely used in traditional medicine to cure various diseases. In this present study, the phytochemical constituents, antioxidant activity and inhibitory effect on alpha amylase by the ethanolic extracts of the *Elaeocarpus ganitrus* (bark) were investigated. The dried bark of *Elaeocarpus ganitrus* was extracted by using ethanol as a solvent for extraction with the help of Soxhlet apparatus. The solvent free crude was screened for presence of phytochemicals, antioxidant activity and alpha amylase inhibitory effect. The screening for phytochemicals showed the presence of carbohydrates, alkaloids, steroids, flavonoids, phenol, tannin, Saponin, glycosides and terpenoids. The ethanolic extract showed a concentration dependent increase in antioxidant activity (Upto 79% by DPPH method and upto 72.8% by H_2O_2 method), which can be used as a potential anti-aging agent and in the treatmentof free radical associated diseases. The ethanolic extract also showed a dose dependent increase in inhibitory effect on alpha amylase (Upto 82%) which can be used as a potential source as antidiabetic drug. From the current study it can be seen that the bark extract of *Elaeocarpus ganitrus* contain diversity of phytochemicals, it is also a potential antioxidant agent and it showed a strong alpha amylase inhibitory activity which may be used as a natural anti-diabetic agent.

INTRODUCTION: In both developed and developing countries diabetes mellitus is a very common and prevalent disease. According to the survey, 25% of the world population is affected by this disease, which is caused by abnormality of carbohydrate metabolism linked to low blood insulin level or insensitivity of target organs to insulin [1]. The plants provide a potential source of antidiabetic drug as many plants and its derivatives have been investigated and proved for its pharmacological effects [2].

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Many plants use alpha amylase inhibitors as a defense mechanism which protects them from insects by altering the digestive action of alpha amylases and proteinases in the gut of insects and inhibit their normal feeding behavior. Thus, alpha amylase inhibitors have potential roles in controlling blood sugar levels [3]. Antioxidants are playing a very important role to reduce oxidative stress which may have the power to damage the biological molecules. The disproportion between oxidants and antioxidant may be responsible for the damage [4].

The genus *Elaeocarpus*, which is having almost 360 species, occurs throughout Australia, India, Nepal, Bhutan, Bangladesh, Malaysia and the Pacific Islands [5]. About 120 species belonging to this genus have been found from different zone of Asia, but in India being the world's top 12 mega

diversity nations out of 120 species, near about 25 species have been reported alone from this zone [6]. *Elaeocarpus ganitrus* Roxb is an evergreen tall tree, ripe fruits and is commonly termed as Rudraksha (In Hindi) in India. It bears a great spiritual, religion and materialistic significance in Hindu mythology. The plant has been traditionally used for the treatment of various ailments like stress, anxiety, depression, nerve pain, epilepsy, diabetes, migraine and lack of concentration etc. [7]. Considering its strong therapeutic significance and extensive use of *Elaeocarpus ganitrus*in Ayurvedic medicine from centuries, it was necessary to evaluate the species phytochemically and pharmacologically in support of its traditional use. With this background information, the present study was aimed to assess the phytochemical constituent, antioxidant property and α-amylase inhibitory potential of the bark of *Elaeocarpus ganitrus.*

MATERIALS AND METHODS:

Collection of Plant Material and Authentication: The bark of healthy plant *Elaeocarpus ganitrus* was collected from local area of Goalpara, Assam in the month of March. The bark was skimmed off with the help of a sharp knife. The plant was authenticated by Botanical Survey of India, Eastern Regional centre, Shillong (No.BSI/ERC/tech/ Identification/2017/559)

Preparation of plant extracts: The collected bark was washed with distilled water to remove the adhering dust particle. Once the water was completely removed, the barks were kept in a shaded place for around 12 days. The dried barks were powdered with the help of the grinder mixture and stored in clean container for further use.

Solvent Extraction: A quantity of 250 grams finely powdered plant materials were placed in Soxhlet apparatus. The extraction was done with ethanol as a solvent. The extraction was carried out for around 20 hours and remaining solvent were evaporated to get solvent free crude extract [8, 9-11].

Test for phytochemicals: Screening for different phytochemicals was done according to the standard manual [4, 12- 15].

Test for Alkaloids: Around 20mgof the ethanolic extract was stirred with diluted HCl (6 mL) on a water bath for 5 mins. These filtrates were divided into three equal and screened for the following reagents:

Dragendorff's test: To one portion of the filtrate, Potassium bismuth iodide solution (Dragendorff's reagent) was added and observed for the presence of an orange red precipitate.

Mayer's test: To the portion of the filtrate, Potassium mercuric iodide solution (Mayer's reagent) was added and observed for the presence of cream colored precipitate.

Wagner's test: Potassium iodide and iodine were dissolved in distilled water and the solution was diluted to 100ml with distilled water. To the remaining part of the filtrate few drops of the above solution was added and observed for the presence of a brown colored precipitate.

Tests for steroids and terpenoids:

Salkowski test: The crude extract was separately shaken with chloroform followed by the addition of concentrated H_2SO_4 along the side of the test tube and observed for the presence of a reddish brown color indicating the presence of steroids.

Liebermann-Burchard test: A part of the extract was mixed with chloroform in a test tube and few drops of acetic anhydride was added to the test tube and boiled in a water bath and rapidly cooled in iced water. To this along the side of the test tube concentrated $H_2SO_4$was added. The test tube was observed for the presence of a brown ring at the junction of two layers and upper layer turning to green shows the presence of steroids and formation of deep red color indicates the presence of terpenoids.

Test for Carbohydrate:

Molisch's Test: Few drops of Molisch's reagent were added to a portion of the sample dissolved in distilled water. To this few drops of H_2SO_4 was added along with the side of the test tube. The mixture was then allowed to stand for two-three minutes and observed for the formation of a red or dull violet colour at the interphase of the two layers.

Benedict's test: The extract was dissolved in distilled water and treated with equal volumes of

Benedict's reagent. The mixture was boiled for 5-10 minutes on water bath and observed for green, yellow or red color.

Test for Protein:

Million test: Around 3 ml test solutions were mixed with 5 ml Million's reagent and observed for white precipitate which on heating turned to brick red.

Ninhydrin Test: Around 2ml of filtrate was treated with few drops of Ninhydrin solution in a test tube which was placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Test for tannins: The extract was mixed with distilled water and then filtered. A few drops of 5% ferric chloride were then added and observed for the presence of black or blue-green coloration or precipitate.

Test for Saponins:

Foam test: 0.5 g of extract was diluted with distilled water to 10 ml shaken vigorously. The formation of frothing which persist on warming in a water bath 5 minutes, suggests the presence of saponins.

Precipitation test: 1ml extract was treated with 1% lead acetate solution. Formation of white precipitate indicates the presence of saponins.

Tests for glycosides:

Borntrager's test: To the extract solution, 5% $H_2SO_4$was added and the mixture was boiled in a water bath and then filtered. The collected filtrate was mixed with equal volume of chloroform and kept aside for 5 min. The lower layer of chloroform was mixed with half of its volume with dilute ammonia and observed for the formation of rose pink to red color of the ammoniacal layer that indicates the presence of anthraquinone glycosides.

Keller-Killiani test: The extract was mixed with distilled water. To this solution glacial acetic acid containing a few drops of ferric chloride was added, followed by few drops of H_2SO_4 along the side of the test tube. This test mixture was observed for the formation of brown ring at the interface that gives positive indication for cardiac glycoside and a violet ring may appear below the brown ring.

Test for Phlobatannins:

Precipitate test: 2 ml of plant extract was boiled with 1ml of 1% aqueous hydrochloric acid and observed for the presence of red precipitation.

Test for Flavonoids:

Alkaline reagent test: A small amount of extract was dissolved in distilled water and treated with few drops of 20% sodium hydroxide solution. This test mixture was observed for formation of intense yellow colour, which becomes colourless on addition of dilute hydrochloric acid that indicates the presence of flavonoids.

Test for Phenols:

Ferric chloride test: A small fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

Antioxidant Property: The antioxidant property was determine by using two methods namely DPPH scavenging activity and Hydrogen peroxide scavenging activity

DPPH Scavenging Activity: The free radical scavenging capacity of ethanolic extract was determined by using DPPH assay according to the previously described method with slight modification [16]. The stock solution of 1 M DPPH was prepared in methanol and kept at 20 ^{0}C until analysis. Fresh 0.1 mM DPPH working solution was prepared by diluting 10 mL stock solution with 90 mL methanol and added to the different concentration of the plant extracts (50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml). The DPPH free radical scavenging was determined in a UV-Vis spectrophotometer (Systronic UV-Vis Spectrophotometre-117) by measuring absorbance at 517 nm against a blank solution by taking ascorbic acid as standard. The scavenging activity was determined by using the following formula:

$$\%Inhibition = \frac{A_{Control} - A_{sample}}{A_{Control}} \times 100$$

Hydrogen Peroxide Scavenging Capacity: The ability of the extracts to scavenge hydrogen peroxide was determined according to the previously standardized method [17]. A phosphate buffer (pH 7.4) was used to prepare a solution of hydrogen peroxide (40 mM).

Different concentration of plant extracts (50µg/ml, 100µg/ml, 150µg/ml and 200µg/ml) were made in distilled water and later added to different test tube containing 600µL hydrogen peroxide solution. After 10 minutes, the absorbance was measured at 230 nm against a blank solution containing the phosphate buffer without hydrogen peroxide. Ascorbic acid was used as a standard. The scavenging activity was determined by using the following formula:

$$\%Inhibition = \frac{A_{Control} - A_{sample}}{A_{Control}} \times 100$$

Alpha-amylase inhibitory activity: By stirring 0.1 g of starch in 100 ml 16mM of sodium acetate buffer, 0.1% (w/v) starch solution was prepared. The enzyme solution was prepared by mixing 27.5 mg of alpha amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5 dinitro salicylic acid solution at 96 mM. Both control and different concentration of plant extracts (100 µg/ml, 200 µg/ml, 300 µg/ml, 400 µg/ml, 500 µg/ml) were added with starch solution and left to react with alpha amylase solution under alkaline conditions at 25 ºC. The reaction was measured for 10 minutes. The generation of maltose was quantified by the reduction of 3, 5 dinitro salicylic acid to 3- amino-5- nitro salicylic acid. The reaction was detected by using 540 nm and inhibition was calculated by the following formula [18, 19]:

$$\%Inhibition = \frac{A_{Control} - A_{sample}}{A_{Control}} \times 100$$

Statistical analysis: Antioxidant and α-amylase inhibition activity were expressed as mean ± standard deviation (SD) of three replicates. Minitab 17 free version was used to calculate the variance of the above parameters.

RESULTS: The ethanolic extract of the bark of *E. ganitrus* was screened for different phytochemicals. The various phytochemicals present in the sample is tabulated in the **Table 1**. The sample showed the presence of various phytochemicals namely alkaloids, flavonoids, steroids, glycoside, phenols and terpenoids.

TABLE 1: TEST FOR PHYTOCHEMICALS

Sl/no	Phytochemical	Ethanol Extracts
1	Carbohydrates	+
2	Proteins	-
3	Alkaloids	+
4	Steroids	+
5	Flavonoids	+
6	Phenol	+
7	Tannins	+
8	Saponin	+
9	Glycosides	+
10	Terpenoids	+
11	Phlobatannin	-

'+' indicates present; '-' indicates absent

Antioxidant assay by DPPH method: Screening of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl(DPPH) method showed a significant percentage of inhibition based on the different concentration. Ascorbic acid was used as a standard and a positive control (OD 0.739) was maintained. The percentage of inhibition is tabulated in the **Table 2**.

TABLE 2: PERCENTAGE OF INHIBITION BY DPPH METHOD AND IC_{50} VALUE

Sl no.	Concentration of sample (µg/ml)	Absorbance(Values represent mean±SD, n=3)	% of Inhibition	IC_{50} value (µg/ml)
1	50	0.423±0.35	42.36%	81.85
2	100	0.337±0.34	54.39 %	
3	150	0.248±0.29	66.44%	
4	200	0.156±0.21	78.89%	

Hydrogen Peroxide method: The antioxidant scavenging activity was also tested by H_2O_2 method and the sample showed significant scavenging activity which is tabulated in the **Table 3**. A positive control was maintained (OD 0.712) and ascorbic acid was used as a standard.

TABLE 3: PERCENTAGE OF INHIBITION BY H_2O_2 METHOD AND IC_{50} VALUE

Sl no.	Concentration of sample(µg/ml)	Absorbance(Values represent mean±SD, n=3)	%of Inhibition	IC_{50} value (µg/ml)
1	50	0.408±0.45	42.7	88.11
2	100	0.343±0.30	51.8	
3	150	0..266±0.48	62.6	
4	200	0.193±0.20	72.8	

***In vitro* alpha amylase inhibition activity:** A dose dependent inhibition of alpha amylase was found by the sample. A positive control (OD 1.096) was carried and percentage of inhibition is tabulated in the **Table 4**.

TABLE 4: *IN VITRO* ALPHA AMYLASE INHIBITION PERCENTAGE AND IC_{50} VALUE

Sl no.	Concentration of sample (µg/ml)	Absorbance (Values represent mean±SD, n=3)	% Inhibition	IC_{50} value (µg/ml)
1	100	0.608±0.44	44.52	167.58
2	200	0.512±0.65	53.28	
3	300	0.425±0.53	61.3	
4	400	0.335±0.55	70	
5	500	0.198±0.32	81.9	

DISCUSSION: Plants are recognized as essential component of the earth's biological diversity which produces necessary resources for the planet. The concept of healing several diseases by medicinal plants has its origin in the antiquity of human civilization. Due to the presence of various bioactive metabolites these medicinal plants are responsible for its various activities and also responsible for producing a definite physiological action on human body. This current study was also targeted to find out the various phytochemical constituents responsible for its various medicinal properties by the plant. As a result of phytochemical screening, the extract showed the presence of various bioactive metabolites like alkaloids, steroids, phenol, flavonoids, tannin, glycoside etc. The medicinal property showed by the sample may be due to the presence of these bioactive metabolites. Upon screening for the free radicals scavenging activity of the sample through standard DPPH & H_2O_2 methods, the sample showed dose dependent scavenging activity upto 79% and72.8% respectively.

The IC_{50} of the extract showed 81.85 µg/ml by DPPH method and 88.11 µg/ml for H_2O_2 method. Hence due to its high level of antioxidant property this plant could be a potential source of natural antioxidant which could be of great importance for the treatment of some radical related diseases, age associated diseases etc. When the ethanolic extract of the bark of *Elaeocarpus ganitrus* was tested for *in vitro* alpha amylase inhibitory effect, the sample showed a concentration dependent inhibitory effect up to 82%. As a result of this, the sample can be used as a potential antidiabetic drug. The sample showed IC_{50} value of 167.58 µg/ml.

Previously available report states the presence of various phytochemicals, antioxidant activity and alpha amylase inhibition property in medicinal plants by various parts like leaves, bark, root *etc.* [3, 9, 11, 19]. This study shows such a trend was apparent with the bark of *Elaeocarpus ganitrus*; though, compound isolation, purification and animal modeling are required to validate the traditional claim.

CONCLUSION: The ethanolic extract of the bark of *Elaeocarpus ganitrus* was found to contain several important bioactive metabolites which are responsible for its various medicinal uses. As the main goal of researchers today is to find out natural antioxidants, this plant may play a crucial role in this field. Due to its high inhibitory effect on alpha amylase, the sample can be used as an alternative for commercially available costly drug. However,

further studies are required to purify compounds and animal studies are required to validate its scientific importance.

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