Phytochemical, Antioxidant Activity And *Invitro* Anti diabetic Study of *Eucalyptus globoidea* Leaf Extracts on Ethylacetate And Ethanol

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Abstract: The present study was designed to evaluate the phytochemical components are present in the leaf of *Eucalyptus globoidea*. The ethyl acetate and ethanol extracts from leaf were prepared and check their phytochemical components revealed the presence of Alkaloids, flavonoids, terpenoids, phenols, and carbohydrates. While saponin and arthroquinone were absent and to assay the antioxidant activity by reducing power assay. Show that ethanol extract of *Eucalyptus globoidea* has significant antioxidant activity the *invitro* antidiabetic activity was studied using the inhibition of α-amylase. The ethanolic extract of eucalyptus *globoidea* has potent antidiabetic activity.

Keywords; Phytochemical , Screening, Antioxidant, Antidiabetic activity,*Eucalyptus Goboidea.*

1. INTRODUCTION:

Medicinal plants are a precious heritage for humanity our ancestors used these plants to ensure their health and transmitted their knowledge and their experiences generation to generation. About 80% of the world population uses this mode of therapy.[3] The WHO has defined traditional medicine as comprising therapeutic practices that have been in existence for hundreds of year.[18]

Medicinal plants are the local heritage with global importance and the world is endowed with a rich wealth of medicinal plants. These plants have made a good contribution to the development of ancient material medical.[19]They are an important therapeutic aid for various awful diseases. In India, from ancient times, different plants of medicinal origin have been used as sources of food from generation to generation and also have good biological properties without any side effects.[1] Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional systems.[17]

Antioxidants are substances that delay or prevent the process of oxidation by scavenging the free radicals in body cells and may reduce potential mutations and thereby help prevent cancer and heart disease.[14, 22] There are many synthetic commercial antioxidants such as butyrate hydroxyl toluene (BHT), butylated

hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gall ate (PG) which are used to reduce the harmful effects of free radicals. However, these synthetic antioxidants may have other harmful effects. Therefore, researching for new antioxidants, especially with natural origin has become a major concern.[21]

According to WHO, the term diabetes mellitus is defined as a metabolic disorder of multiple etiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs.[12]

The conventional treatments for diabetes include the reduction of the demand for insulin, stimulation of endogenous insulin secretion, enhancement of the action of insulin at the target tissues and the inhibition of degradation of oligo and disaccharides .[7] The enzymes α-glucosidase are responsible for the breakdown of oligo and/or disaccharides to monosaccharides. The inhibitory action of these enzymes leads to a decrease of blood glucose level, because the monosaccharides are the form of carbohydrates which is absorbed through the mucosal border in the small intestine.

Another effective method to control diabetes is to inhibit the activity of α-amylase enzyme which is responsible for the collapse of starch to more simple sugars dextrin, maltose and glucose.[2] This is contributed by α-amylase inhibitors, which delays the glucose absorption rate thereby maintaining the serum blood glucose in hyperglycemic individuals.[6] Some inhibitors currently in clinical use are acarbose and miglitol which inhibit glycosidases such as α-glucosidase and α-amylase while others such as and voglibose inhibit α-glucosidase.

2. MATERIALS AND METHODS

2.1 COLLECTION OF PLANT MATERIALS

The fresh leaves of *Eucalyptus globoidea* were collected randomly from the yercaud Hills, Tamil Nadu. Sample materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles in the refrigerator

2.2 PREPARATION OF EXTRACTS (Soxhlet Method):

Crude Sample extract was prepared by Soxhlet extraction method. About 20gm of powdered sample material was uniformly packed into a thimble and extracted with 250ml of different solvents like ethanol and ethyl acetate separately. The process of extraction has to be continued for 24 hours or till the solvent in siphon tube of extractor become colorless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºc till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºc till future use.

2.3 PHYTOCHEMICAL SCREENING (Brain & Turner 1975)

Preliminary phytochemical analysis was carried out by ethanol and ethyl acetate extracts of *Eucalyptus globoidea* as per standard methods.[4]

2.3.1 Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrate was used.

Mayer's Test: Filtrates were treated with Mayer's reagent. Formation of a yellow cream precipitate indicates the presence of alkaloids.

2.3.2 Detection of Flavonoids

Lead Acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

2.3.3 Detection of Steroids

Liebermann- Burchard Test: 2ml of acetic anhydride was added to 0.5g of the extracts, each with 2ml of H_2SO_4. The color changed from violet to blue or green samples indicate the presence of steroids.

2.3.4 Detection of Terpenoids

Salkowski's Test: 0.2g of the extract of the whole plant sample was mixed with 2ml of chloroform and concentrated H_2SO_4 (3ml) was carefully added to form a layer. A reddish brown coloration of the inner face was indicates the presence of terpenoids.

2.3.5 Detection of Anthroquinones

Borntrager's Test: About 0.2g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% NH_3 were added to the mixture and heated. Formation of pink colour indicates the presence anthraquinones

2.3.6 Detection of Phenols

Ferric Chloride Test: Extracts were treated with few drops of 5% ferric chloride solution. Formation of bluish black color indicates the presence of phenol

2.3.7 Detection of Saponins

Froth Test: About 0.2g of the extract was shaken with 5ml of distilled water. Formation of frothing (appearance of creamy stable persistent of small bubbles) shows the presence of saponins.

2.3.8 Detection of Tannins

Ferric Chloride Test: A small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and 0.1% ferric chloride was added to the filtrate. A dark green color formation indicates the presence of tannins.

2.3.9 Detection of Carbohydrates

Fehling's Test: 0.2gm filtrate is boiled on water bath with 0.2ml each of Fehling solutions A and B. A red precipitate indicates the presence of sugar.

2.4 Reducing Power Assay (Oyaizu, 1986).

The ethanol extract solutions were spiked with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was kept in a 50°C water-bath for 20min. The resulting solution was cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid, and centrifuged at 3000rpm for 10min. The supernatant (5ml) was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride and incubated for 10min. The absorbance was detected at 700nm on spectrophotometer. The extract concentration providing the absorbance was calculated from the graph of absorbance at 700nm against extract concentration. Ascorbic acid was used as standard. Higher absorbance indicates higher reducing power.

2.5 In vitro method employed in Anti-Diabetic studies (Hamdan and Afifi, 2004)

2.5.1 Inhibition of alpha amylase enzyme

A total of 500 µl of ethanol extract samples drug (100-1000µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 donator salicylic acid color reagent.

The test tubes were incubated and then in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10ml distilled water and absorbance was measured at 540nm. Control represents 100% enzyme activity and was conducted in similar way by replacing extract with vehicle. [20]

2.5.2 Inhibition of Alpha Glucosidases Enzyme

The inhibitory activity was determine a solution of starch substrate (2 % w/v maltose or sucrose) 1ml with 0.2 M Tris buffer pH 8.0 and various concentration of plant extract for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method.[4]

2.5.3 Calculation of 50% Inhibitory Concentration (IC_{50})

The concentration of the plant extracts required to scavenge 50% of the radicals (IC_{50}) was calculated by using the percentage scavenging activities at five different concentrations of the extract. Percentage inhibition (I %) was calculated by I % = (Ac-As)/Ac X 100, where Ac is the absorbance of the control and as is the absorbance of the sample.[16]

3. RESULT AND DISCUSSION.

3.1 YIELD OBTAINED

The yield for ethanol and ethyl acetate extract of *Eucalyptus globoidea* were obtained. (Table.1) The yield obtained in the ethanol extract was higher when compared to the ethyl acetate extract. Thus, further analysis was carried out using the ethanol extract of a *Eucalyptus globoidea*.

Table: 1 Yield Obtained In *Eucalyptus globoidea*

S. No	Ethanol extract	Ethyl acetate extract
1	25.31	12.96

3.2 PHYTOCHEMICAL ANALYSIS

In the present study, the preliminary phytochemical screening of ethyl acetate and ethanol extract of *Eucalyptus globoidea* leaves were done (Table.2)

Table: 2 QUALITATIVE PHYTO CHEMICAL ANALYSIS OF *Eucalyptus globoidea*

Phytochemical	Observations	Extracts	
		Ethanol	Ethyl acetate
Alkaloids Mayer's Test	Cream color	+	-
Flavonoids Lead acetate Test	Yellow orange	+	+
Steroids Liebermann-Burchard Test	Violet to blue or Green color formation	-	+
Terpenoids Salkowski Test	Reddish brown precipitate	++	+
Arthroquinone Borntrager's Test	Pink color	-	-
Phenols Ferric chloride Test	Deep blue to Black color formation	++	+

Saponin	Stable persistant	-	-
Tannin	Brownish green / Blue black	-	-
Carbohydrates	Yellow / brownish / blue / green color	++	+
Oils & Resins	Filter paper method	-	+

* + = PRESENCE, - = ABSENCE

Phytochemical analysis of the ethanol extract of *Eucalyptus globoidea* leaves showed in the (Figure.4) presence of Alkaloids, Flavonoids, terpenoids and Phenols. The ethyl acetate extract of *Eucalyptus globoidea* leaves showed in the (Figure.5) presence of flavonoids, steroids, terpenoids, phenol and oil & resins. Carbohydrates were present in both the extract of *Eucalyptus globoidea*. In the result of phytochemical analysis, the higher activity was seen in the ethanol extract of *Eucalyptus globiodea*.

Tannins in their mechanism of anthelmintic action are known to interfere with energy Generation by uncoupling oxidative phosphorylation or they may interfere with glycoprotein of cell surface. Tannins can also react with nematode's cuticle and toughens the skin. Alkaloids act on central nervous system and caused paralysis of the worms. Flavonoids have a number of hydroxyl groups connected with the aromatic which enhance toxicity to the worms and ant oxidative effectives[10, 13, 9, 11].

3.3 Antioxidant Activity

The antioxidant activity of ethanol extract of *Eucalyptus globoidea* was evaluated by reducing power assay. It was observed that the antioxidant activity was directly proportional to the concentration of the extract. Thus, it can be inferred from the results that the ethanol extract of *Eucalyptus globopidea* has significant antioxidant activity (Table No3).

Eucalyptus grown here in Adigrat has showed to have antioxidant potency. However, compared to their anthelmintic activity they were less potent. The presence of some of the secondary metabolites responsible for antioxidant activity could be a clue to the recorded percentage inhibition. On the other hand, the less abundance in most of them and the absence of phenol can be the reason to the decrements in activity[38].

Table: 3 Reducing power Assay

S.No	Concentration *µl*	Ethanol absorbance at (700nm)	IC_{50}	% IC_{50}
1	50 µl	0.184	25.17	693.35
2	250 µl	0.193	31.29	
3	500 µl	0.216	46.93	
4	750 µl	0.224	52.38	
5	1000 µl	0.235	59.86	

3.4 Antidiabetic studies *Invitro* Methods:

The in vitro antidiabetic activity of the ethanol extract of *Eucalyptus globoidea* (Table.4) was studied using the inhibition of alpha amylase enzyme to the concentration of the ethanol extract of *Eucalyptus globoidea*. The %IC50 of was found to be 582.43µg/ml. Therefore the ethanol extract *of Eucalyptus globoidea* has potent antidiabetic activity.

The medicinal plants or natural products involve retarding the absorption of glucose by inhibiting the carbohydrate hydrolyzing enzymes .Several α-amylase inhibitors including acarbose, voglibose and miglitors are clinically used for treatment but their prices are high and clinical side effects occur. [15]The in vitro anti diabetic activity of the ethanol extract of *Eucalyptus globoidea* (Table.5) was studied using the inhibition of alpha glucosidases enzyme to the concentration of the ethanol extract of Eucalyptus globoidea. The % IC50 of was found to be 325.61µg/ml. Therefore the ethanol extract of *Eucalyptus globoidea* has potent antidiabetic activity.

The modulation of carbohydrate metabolizing enzymes activity has been proclaimed as a therapeutic modality of choice in controlling postprandial hyper glycemic[4]. Pancreatic α-amylase hydrolyses starch into oligosaccharides and disaccharides, while intestinal α-glucosidase aids final conversion of disaccharides to simple sugars inform of glucose. The glucose liberated from these catabolic events is subsequently absorbed via the intestinal epithelia into systemic circulation. Hence, the inhibition of these enzymes activities normalizes carbohydrate metabolism, thereby extenuating postprandial hyperglycemia in Diabetics. Plant-derived α-amylase and α-glucosidase inhibitors with excellent antioxidative potentials offer attractive alternative in this regard.[8]

Hence screening of α-glucosidase inhibitors from plants and synthetic sources is increasing and inhibitors of these enzymes have been recently developed from natural sources[36]

Table:4 Inhibition of Alpha Amylase Enzyme

S.No	Concentration µl	Ethanol absorbance at (700nm)	IC_{50}	% IC_{50}
1	50 µl	0.208	36.84	582.43
2	250 µl	0.214	40.79	
3	500 µl	0.221	45.39	
4	750 µl	0.234	53.95	
5	1000 µl	0.248	63.16	

Table: 5 Inhibition of Alpha Glucosidases Enzymes

S.No	Concentration µl	Ethanol absorbance at (700nm)	IC_{50}	% IC_{50}
1	50	0.217	42.76	325.61
2	250	0.225	48.03	
3	500	0.238	56.58	
4	750	0.242	59.21	
5	1000	0.249	63.82	

4. SUMMARY AND CONCLUSION

In the present study, *Eucalyptus globoidea* plant was studied for the phytochemical constituents, antioxidant and *invitro* antidiabetic activities. The qualitative analysis of Phytochemical was done in the ethyl acetate and ethanol extracts of *Eucalyptus globoidea* leaves. The results showed the presence of Alkaloids, Flavonoids, Terpenoides, Phenols and carbohydrate in the ethanol extract whereas flavonoids, steroids, Terpenoides, Phenols, carbohydrate, oils and resins were present in the ethyl acetate extract.

The antioxidant activity of the ethanol extract of *Eucalyptus globoidea* was studied with the help of reducing power assay. And the reducing power was found to be concentration dependent.

The in vitro anti diabetic studied inhibition of alpha amylase enzyme was found in the increase concentration of the ethanol extracts of *Eucalyptus globoidea*.

From the above results, it can be concluded that the of ethanol extracts *of Eucalyptus globoidea* possesses significant phytochemical constituent and therefore has potent antioxidant and anti diabetic activity therefore specific compounds can be isolated from the plant extract to treat various diseases.

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