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Phytochemical and *In Vitro* Antioxidant Evaluation of Ethyl Acetate Extract of *Guazuma ulmifolia* Fruit

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Abstract
To analyse quantitive phytochemical and *in vitro* antioxidant properties of ethyl acetate fruit extracts of *Guazuma ulmifolia*. Quantitative phytochemical analysis for total phenolic, flavonoids, alkaloids and tannins were made by following standard procedures. Antioxidant potential ethylacetate fruit extracts of *Guazuma ulmifolia* were studied by using different in vitro model like DPPH, FRAP, ABTS, and the scavenging activity was investigated by the production of nitric oxide, superoxide anion scavenging activity, hydrogen peroxide by adapting standard methods. The quantitative phytochemical analysis of this *Guazuma ulmifolia* fruit exhibited the presence of alkaloids, total pheniolic, flavonoids and tannins in considerable quantity. In vitro antioxidant activity of the Guazuma ulmifolia show prominent antioxidant properties.

Keywords
Guazuma ulmifolia, ethylacetate, phytochemical, antioxidant.

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INTRODUCTION
Medicinal plant has been used in different part of the world to treat human disease and infection. Plants are used as medicine early in different countries and their source of much potential and powerful drug traditional medicine using plant extract continue to provide health coverage for over 80 percentage of the world population especially in the developing world [1]. Drugs from the plant are easily available, safe, efficient less expensive and have less Side Effects. Natural products of higher plant possess a new source of antimicrobial agent and antioxidant agent with possibly novel mechanism of action.
Medicinal plant represents a treasure trove of structurally diverse bioactive molecules. Now a day's these bioactive phytochemicals play a vital role against number of diseases such as cancer, asthma, stroke, diabetes, etc. unlike pharmaceutical chemicals these phytochemicals do not have any facet effects. Since the phytochemicals cure disease without causing any harm to human beings due to the presence of rich antioxidant can also considered as "man friendly medicines". Therapeutical actions of

plants are unique de the presence of secondary metabolites such as teroids, tannins, Qinines, terpenoids and flavonoids [2],were rich in antioxidant compounds to protect protect cells against the damage caused by reactive oxygen species, such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite[3], which results in oxidative stress leading to cellular damage. Free radicals are chemical entities that exist with one or more unpaired electrons. The propagation of free radicals can bring about many adverse reactions leading to extensive tissue damage, lipids, proteins and DNA are all susceptible to attack by free radicals [4]. Resistance against oxidative stress by scavenging the free radicals.is done by antioxidants that can reduce or inhibits the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions. Phenolic compounds antioxidant activity is mainly due to their redox properties, reacting to neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides.

Common name of *Guazuma ulmifolia* is West Indian elm or bay cedar, is a medium-sized tree normally found in pastures and disturbed forests. This flowering plant from the Malvaceae family grows up to 30m in height and 30–40 cm in diameter. It is normally found in the Caribbean, Mexico, Central America and Colombia, Ecuador, Peru, Bolivia, Paraguay, Argentina, and Brazil. In India it has been cultivating them or more than 100 years. It flowers throughout the year, in particular from April to October. A beverage of crushed seeds soaked in water is employed to treat diarrhea, dysentery, colds, coughs, contusions, and venereal disease. Its seeds also used as a diuretic and astringent [5].

The present study aims to explain the quantitative and antioxidant capacity of ethylacetate fruit extract of Guazuma ulmifolia.

MATERIAL AND METHODS

Collection of plant material

Fruits of *Guazuma ulmifolia* was collected from valluvar kottam, Nungambakkam in Tamil Nadu. The fruits were washed thoroughly with tap water to remove dust particles and other unwanted impurity. Then it was shade dried for 6 to 7 days and coarsely powdered separately and stored in well closed bottles for further analysis in laboratory.

Extraction Procedure

The fruit with seed of *Guazuma ulmifolia* was carfully grinded and coarsely powdered. The powder was subjected to solvent extraction with Ethyl actate. The Extracts was concentrated by using the Rotary Evaporator and the yield the Extract was collected and stored for future analysis.

QUANTITATIVE ANALYSIS OF FRUIT EXTRACT OF *Guazuma ulmifolia*

Estimation of tannins content

The total Tannins content of *Guazuma ulmifolia* was estimated by treating with 100 mg of polyvinyl polypyrrolidone and 500 µL of distilled water. This solution was incubated at 4 °C for 4 h. Then the sample was centrifuged at 5 000 r/min for 5 min and 20 µL of the supernatant was taken. This supernatant has only simple phenolics free of tannins the tannins would have been precipitated along with the polyvinyl polypyrrolidone[6]. The tannin content of the supernatant was measured at 725 nm and expressed as the content of free phenolics on a dry matter basis.

Determination of total flavonoid content

The total flavonoid content of *Guazuma ulmifolia* the fruit extract was determined by the Aluminum chloride colourimetric method [7]. 200 µl of fruit extract was taken in a test tube and the solvent was allowed to evaporate, then 5 ml of 0.1 M aluminum chloride was added and shaken well, and incubation for 40 min at room temperature and the absorbance was measured at 415 nm using UV–visible spectrophotometer. The total flavonoid content expressed as milligrams of Quercetin equivalent per gram of dry weight (mg QE/g DW) of the plant material using standard plot of quercetin.

Determination of alkaloids

The fruit extract (1mg) was dissolved in dimethyl sulphoxide (DMSO), added 1ml of 2 N HCl and filtered. This solution was transferred to a separating funnel; 5 ml of bromocresol green solution and phosphate buffer were added. The mixture was shaken with chloroform and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. The absorbance value was found for 470 nm using UV–Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/g of extract [8] using standard plot of atropine.

IN VITRO ANTIOXIDANT ANALYSIS OF FRUIT EXTRACT OF *Guazuma ulmifolia.*

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Free radical scavenging assay for ethyl acetate extract of *Guazuma ulmifolia was* taken at various concentrations 100 to 500 µl /ml, in small tubes and made up to1 ml using methanol. 1 ml of 0.01 mM DPPH dissolved in methanol was added to all the test concentrations and maintained in the dark for 30 min, at room temperature. The absorbance value was found for 517 nm. The percentage inhibition and the IC_{50} values were calculated with DPPH as the

control and butylated hydroxyanisole BHA as the reference [9]. The concentration in µg of dry material per ml of solvent (µg/ml) that inhibits the formation of DPPH radicals by 50% is defined as IC_{50} value.

DPHH scavenging activity (%) = (A_0–A_1) /A_0×100

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Ferric reducing antioxidant ability assay

FRAP assay1 ml of plant extract, 2.5 ml phosphate buffer of 0.2 M, pH 7 and 1% potassium ferricyanide 2.5 ml were mixed and incubated at 50◦C for 30 min, then 2.5 ml of 10% trichloroacetic acid was added and centrifuged at 6500 rpm for 10 min. Distilled water 2.5 ml and 0.5 ml of 0.1% FeCl3were added to 2.5 ml of the supernatant. The absorbance value of the solution was measured at 700 nm using UV–visible spectrophotometer [10].

FRAP scavenging activity (%) = (A_0 –A_1) /A_0×100

Where, A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

ABTS radical scavenging activity

ABTS radical cation was generated by oxidation of ABTS 7 mmol/L with potassium persulfate 2.45 mmol/L which was dissolved in 5 mL of distilled water [11]. After incubation for 12-16 h at room temperature in dark condition, blue/green ABTS chromophore was produced. The ABTS solution was diluted with ethanol 1:89 v/v and adjusted to equilibrate the absorbance of 0.700±0.001 at 734 nm. The generated $ABTS^{\bullet+}$ solution 2 mL was mixed with 20 µL of sample extracts or trolox standards 0-15 µmol/L, after30 minutes absorbance values were read at 734 nm. The inhibition percentage of ABTS radical was calculated using the following formula:

ABTS scavenging activity (%) = (A_0 –A_1) /A_0×100

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample.

Reducing power assay

The fruit extract in 1ml of methanol at various concentrations was mixed with a phosphate buffer 5 ml, 0.2 M, pH 6.6 and potassium ferricyanide 5 ml, 1%, and the mixture was incubated at 50°C for 20 min. Next, 5ml of trichloroaceticacid 10% were added to the reaction mixture, which was then centrifuged at 3000 RPM for 10min. The upper layer of the solution 5 ml was mixed with distilled water 5ml and ferricchloride 1 ml, 1%, and the absorbance was measured at 700 nm. A stronger absorbance will indicate increased reducing power [12].

Hydrogen peroxide scavenging activity

The reaction mixture contained deoxyribose 2.8 mM, KH_2PO_4–NaOH buffer, pH 7.4 0.05 M, $FeCl_3$ 0.1 mM, EDTA 0.1 mM, H_2O_2 1 mM, ascorbate 0.1mM ethyl acetate extract of *Guazuma ulmifolia fruit was* taken at various concentrations 100 to 500 µl /ml in a final volume of 2 mL. The reaction mixture was incubated for 30 min a followed by addition of 2 mL of trichloroacetic acid (2.8% w/v) and thiobarbituric acid. The reaction mixture was kept in a boiling water bath for 30 min, cooled and the absorbance was [13] read at 532 nm in a UV spectrophotometer and percentage of inhibition was calculated using the same formula as DPPH.

Nitric oxide scavenging activity:

Nitric oxide radicals were produced from sodium nitroprusside solution and measured by the Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide radicals which interfere with oxygen to produce nitrite ions. Scavengers of nitric oxide act against oxygen, leading to reduce production of nitrite ions. About 3 mL of sodium nitro prusside 10 mmol/L in phosphate buffer saline 0.2 mmol/L, pH 7.4 was added with various concentrations of the extracts 250-450 µg/mL and it was incubated at 25 °C for 150 min. Then 500 µL of Griess reagent 1% sulphailamide, 2% orthophosphoric acid, 0.1% N-1-napthylethylenediamine dihydrochloride was added [14]. The absorbance values were measured at 546 nm and percentage of inhibition was calculated using the same formula as DPPH

RESULT AND DISCUSSION

Total tannins content

The total tannin were expressed as mg GAE/100 g, tannic acid equivalent using the standard curve equation, y=0.001x-0.064, R^2 =0.988, where y is absorbance at 725nm and x is the total tannin.The gravimetric analysis for total tannin contents in *Guazuma ulmifolia* fruit extract is 224.44 mg/500g. The presence of tannin is *Guazuma ulmifolia* fruit will help to protect from different diseases, disorders caused by microbial infections and potent free radicals scavenging effect.

Total flavonoids content

The total flavonoids were expressed as QE/g DW, Quercetin equivalent using the standard curve equation, y=-0.001x-0.086 where y is absorbance at 415 nm and x is the flavonoids content.The gravimetric analysis for total flavonoids contents in *Guazuma ulmifolia* fruit extract have greater flavonoids , 332 mg/500 g than the total tannin and alkaloids content. These flavonoids help to induces mechanism that kills cancer cell, inhibit tumor invasion, apart from this show antiallergic, anti-inflammatory, antimicrobial and anticancer.

Table 1: Quantitative phytochemical analysis in *Guazuma ulmifolia* fruit extract.

Concentration	Tannin content	Flavonoid content	Alkaloids content
100	52.22	63.65	36.36
200	63.33	108.98	90.90
300	107.77	149.96	136.36
400	141.11	213.54	200.00
500	224.44	314.34	290.90

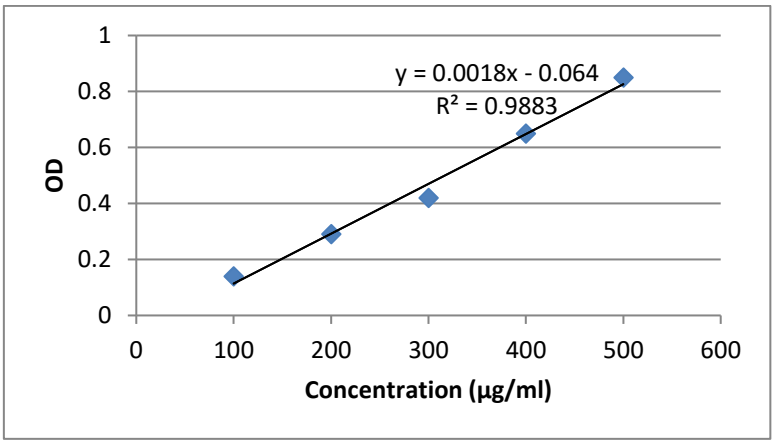

Figure 1: Linear regression of Total Tannin content

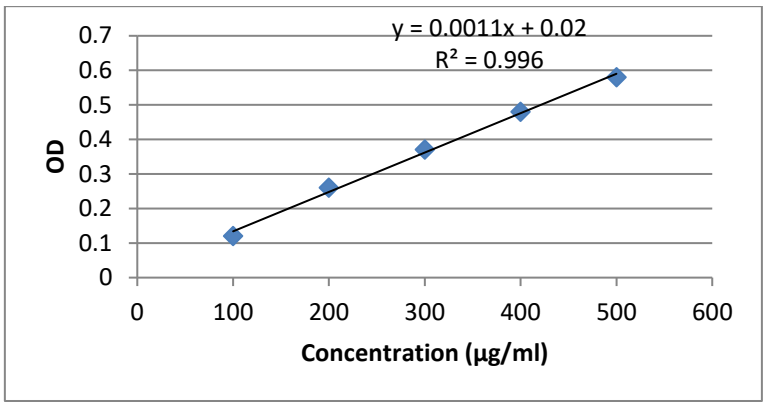

Figure 3: Linear regression of total alkaloids content

Table 2: Antioxidant activities of ethyl acetate extract of *Guazuma ulmifolia* fruit

concentration (mg/ml)	Inhibition % of DPPH	Inhibition % of FRAP	inhibition % of NO	inhibition % of H_2O_2	inhibition % of ABTS	inhibition % of SO
100	3.8	21	0.1	1.0	31.8	4
200	15.3	48	5.5	7.8	47.8	12
300	19.2	51	11.1	15.7	65.2	24
400	23	61	16.6	21	84	32
500	40.3	70	16.6	28.9	86.9	40
IC_{50}	6.677mg/ml	2.981mg/ml	12.038mg/ml	7.974mg/ml	2.102mg/ml	5.565mg/ml

Determination of alkaloids

The total alkaloids were expressed as mg of AE/g ,atropine equivalent using the standard curve equation, y=0.001x+0.02, R2 =0.996, where y is absorbance at 765nm and x is the total alkaloids.The gravimetric analysis for total alkaloid contents in *Guazuma ulmifolia* fruit extract have higher alkaloids, 290.90 mg/500 g sample) than the total tannin content. These alkaloids have antitumour,

immune suppressant, insecticidal and antifeedal properties. It also protects against chronic disease.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay

Antioxidant scavenging property of the extract was studied by using DPPH activity. By increasing in the concentration of extract there is decrease the DPPH radicals by IC50, which is a parameter widely used to estimate the antioxidant activity [15]. DPPH is a stable free radical and that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. A freshly prepared DPPH solution is of deep purple colour with absorption maximum at 517 nm and in the presence of antioxidant, this colour disappears due to quenching of DPPH free radicals and converting them into a colourless product 2,2-dipenyl-1-picryl hydra-zine,Hence DPPH is usually used as a substance to evaluate the antioxidant activity. The ethylacetate extract of *Guazuma ulmifolia* fruit were examined for antioxidant activity by DPPH method for five different concentrations were analysed, scavenging effects on the DPPH radical which was increasing with the increase in the concentration of the sample .% inhibition for 500µg/ml is 40.3 is high than other four concentration, IC50 6.677mg/ml. This might be due to the presence of higher flavonoids content. The study showed that the fruit extract has the proton donating ability and could serve as free radical scavengers, acting possibly as primary antioxidant.

Antioxidant activity of *Guazuma ulmifolia* fruit by FRAP assay

Ferric reducing antioxidant ability FRAP is a novel method for assessing antioxidant power as reductants in a redox –linked colorimetric method employing an easily reduced oxidant [16]. The reducing ability of a compound is due to reductants, which exhibit antioxidant potential by breaking the free radical chain with the donated hydrogen atom which reduce ferric tripyridyltrizine from a colourless complex ferrous to 2,4,6-tripyridyl-s-triazine, absorption at 593nm readings are related to the reducing power of the electron donating antioxidants present in the test compound. % inhibition of FRAP is less high than ABTS antioxidant assay, IC_{50} 6.677mg/ml.

Antioxidant activity of *Guazuma ulmifolia* fruit by NO assay

Nitric oxide is an important chemical generated by endothelial cells, macrophages, neurons, etc. and is involved in the regulation of various physiological process. Nitric oxides formed during their reduction with oxygen or with superoxides such as NO_2, N_2O_4, N_3O_4 are very reactive. The excess concentration of NO will cause diseases in human beings which can alter the structural and functional behaviour of many cellular components. Nitrite ions react with Griess reagent and form a purple azo dye [17]. The decrease in the formation of purple azo dye reflects the presence of free radicals scavengers in the fruit compounds. From its IC_{50} is 12.03837 mg/ml it is clear that *Guazuma ulmifolia* fruit has a noticeable effect to scavenge nitric oxide radicals, which may be due the presence of phenolic compound with potent free radical-scavenging effect.

Antioxidant activity of *Guazuma ulmifolia* fruit by H2O2 assay

The hydroxyl radical H_2O_2 scavenging activity is measured as the % inhibition of hydroxyl radical generated in Fenton's reaction mixture by studying the competition between deoxyribose and the extract for hydroxyl radicals generated from Fe^{3+} /ascorbate/EDTA/H_2O_2 system. The hydroxyl radical attacks deoxyribose which eventually results in TBARS formation. Ferrous salts can react with H_2O_2 and forms hydroxyl radical via Fenton's reaction. The iron required for this reaction is obtained from the pool of iron of HEME containing proteins [18]. The hydroxyl radical thus produced may attack the sugar of DNA base causing sugar fragmentation; base loss and DNA stand breakage. For500µg/mL concentration % inhibition of H2O2, ethylacetate extract of *Guazuma ulmifolia* fruit is 28.9, IC_{50} is 7.974mg/ml

Antioxidant activity of *Guazuma ulmifolia* fruit by ABTS assay.

The reduction of the 2, 2'azinobis (3-ethylbenzothiazoline sulphonate) radical cation ABTS has been widely used to measure the antioxidant capacity of natural extracts [19]. A stable free radical with the characteristic absorbance at 734 nm, was used to study the radicals scavenging effect of ethyl acetate extract of *Guazuma ulmifolia* fruit.The presence of bioactive chemical compounds in the fruit extracts that inhibit the potassium persulfate activity may reduce the production of ABTS. This study reveals ethyl acetate extract of *Guazuma ulmifolia* fruit exhibited highest ABT radical scavenging activity when compared with another antioxidant activity assy, IC_{50} is 2.102459mg/ml

Antioxidant activity of *Guazuma ulmifolia* fruit by SO assay.

superoxide anion is very harmful to cellular components, reported that flavonoids are effective antioxidants mainly because they scavenge superoxide anions. The superoxide radical scavenging activities of the fruit extract were increased markedly with increasing concentrations. The results of IC_{50} is grater then 5mg/ml.

CONCLUSION

It can be concluded that ethylacetate extract of *Guazuma ulmifolia* fruit has effective in scavenging free radicals and has the potential source of natural antioxidants and this justified its uses in folkloric medicines. On the basis of response in terms of scavenging radicals and reducing power activity, it is concluded that, *Guazuma ulmifolia* fruit possessed potential antioxidant activity. It may be due to the presence of respective secondary metabolites such as phenolics, flavonoids, tannins, cardiac glycosides, Terpenoids, steroids etc.in the species. The strong correlation between the contents of total phenolics, flavonoids and alkoloidsand radical scavenging activity indicates that these phytochemical constituents are major contributors to the antioxidant potential of this species. The presence of various phytochemicals in the tested plant reveals that this plant may be a good source for production of new drugs for various ailments.

Therefore, this species can be attempted to derive the drugs of antioxidant properties. However, further studies need to be carried in the isolation of the active compound *Guazuma ulmifolia* fruit. It further reflects a hope for the development of novel anticancer agents

CONFLICT OF INTERESTS

We declare that there were no conflicts of interest.

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