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Phytochemical, Antioxidant and Cytotoxic Activity of Leaf Extracts of *Turnera ulmifolia*

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ABSTRACT

Turnera ulmifolia, a flowering plant was explored for its medicinal potential using phytochemical tests, cytotoxic and antioxidant activities. Four solvent extracts namely hexane, chloroform, ethyl acetate and methanol extracts were prepared from the leaves. On phytochemical analysis, all the extracts showed two important class of phytochemicals i.e; flavonoids and terpenoids. The ethyl acetate and methanol extracts also possessed tannins and phlorotannins. Cytotoxic activity was best in methanol extract (>80%) followed by ethyl acetate extracts which exerted 72% activity at a higher dose of 200microgram/ml. Other two extracts were mildly active. Highest scavenging property against DPPH radical was estimated in ethyl acetate extract with IC50 value 251.767µg/ml ranging from 250µg/ml to 500µg/ml. same showed the best activity in FRAP assay as well. Nitric oxide scavenging property was maximum in methanolic extract of *Turnera ulmifolia*. Overall it can be concluded that a lesser known species *Turnera ulmifolia* exhibited significant cytotoxic as well as antioxidant potential.

INTRODUCTION

Turnera ulmifolia is a perennial shrub belonging to family Turneraceae. A number of species of the genus have been reported to be useful in the treatment of anemia, bronchitis, cough, diabetes, fever, fungal disease, pain, pulmonary and respiratory disease, skin disorders, abortive, expectorants, laxative and women's health problems[1]. It is prescribed in chest ailments, biliousness, indigestion and rheumatism [2]. In java, an infusion of the leaves is stated to be used against dysentery. The leaves contain sterols to be used against dysentery [3]. *Turnera diffusa* of this genus with a strong odour possess essential oil. Leaves of the same are used as tea which has a relaxing kind of effects and are considered aphrodisiac [4]. A number of compounds have been isolated from *T. diffusa*, some of these are arbutin, flavonoids, phenolics and sesquiterpenoids[5].

As a number of phytochemically important compounds have been reported from the genus, it was considered worthwhile to explore the biological properties of the shrub *Turnera ulmifolia.* Thus solvent leaf extracts were subjected to phytochemical tests, cytotoxic and antioxidant potential. As all these biomarkers are good indicators of medicinal potential of the plants.

MATERIALS AND METHODS

Collection and processing of plant material

Leaves of *Turnera ulmifolia* were collected from the medicinal germplasm garden of Regional Plant Resource Centre, Bhubaneswar, Odisha. After drying the leaves material was grinded well using a mechanical blender to a fine powder and transferred into airtight containers with proper labeling for future use. Solvent extracts were prepared by Soxhlet extraction method. About 20gm of powdered plant material was uniformly packed into a thimble and extracted with 250ml of different solvents separately. Solvents used were hexane, chloroform, ethyl acetate and methanol. The process of extraction continues for 24-48 hours. After extraction, the extract was concentrated Buchi rotavapor under vacuum and controlled temperature of 45ºC-50ºC. The dried extract was stored in screw capped vials and kept in a refrigerator at 4ºC for further use.

Phytochemical tests

Phytochemical analysis for the presence of alkaloids, saponins, tannins, glycosides and terpenoids were conducted using the standard protocols [6, 7].

Brine shrimp lethality test:-

Brine shrimp **(*Artemia salina*)** eggs were incubated for 48hrs (3.6gm of black salt in 200ml distil water) to get the desired growth of the larvae for biological evaluation. Stock solutions of different extracts were prepared at a concentration of 10µg/µl. The cytotoxic assay was carried out at five doses 25, 50,100, 200 and 400µg/ml, for each dose level 3 replicates were used. Motility, readings were taken every hour up to 4hrs. Motility was graded. After 24hrs the final reading was taken and percentage of inhibition was calculated by comparing the treated samples with the controls. The standard deviation was also calculated.

Antioxidant activity (Qualitative screening of antioxidants):

DPPH Assay

To detect antioxidant activity, qualitative 2, 2 - Diphenyl1-picrylhydrazyl (DPPH) assay was carried out. The plates were first air-dried and then the chromatograms were sprayed with 0.2% DPPH in methanol as an indicator [8]. The presences of antioxidant (AH) compounds were detected by yellow spots against a purple background on the TLC plates sprayed with 0.2%DPPH in methanol.

$DPPH + AH \rightarrow DPPH - H + A^{-}$

(Purple color) (Yellow color)

Qualitative screening of the constituents in each of the leaf extracts of *Turnera ulmifolia for* antioxidant activity was done by TLC analysis. The process was carried out using TLC sheets. For about 5µl of each sample was loaded on the TLC sheet and the chromatograms were developed in following solvent systems:

a) Ethyl acetate: methanol: water (40:5.4:4) [EMW] (polar neutral)

b) Chloroform: ethyl acetate: formic acid (5:4:1) [CEF] (Intermediate polarity/acidic)

c) Benzene: ethanol: ammonium hydroxide (90:10:1) [BEA] (Non-polar/basic)

Quantitative antioxidant assay:

DPPH radical scavenging assay:

For DPPH free radical scavenging assay 1mM DPPH **(2, 2- Diphenyl-1- picrylhydrazyl)** (Mol. Wt. 394.33) solution was prepared. 4mg DPPH was weighed and dissolved in 10ml methanol. DPPH assay was done by serial dilution method starting from concentration 5000µg, 2500µg, 1250µg, 625µg, 312.5µg, 156.25µg, 78.13µg, 39.06µg, 19.53µg, 0.976µg. 500µl DPPH solution was added to each test tube and stirred thoroughly before incubated for 30min. Then optical density (OD) was measured at λ = 517nm in a spectrophotometer. The percentage radical scavenging activity was calculated from the following formula:

% Scavenging [DPPH] = [(Ao - A1) / Ao] * 100

Where Ao was the absorbance of control and A1 was the absorbance of the sample.

Nitric oxide radical scavenging Assay: Sodium nitroprusside 5 mM was prepared in phosphate buffer (pH 7.4). To 1 ml of various concentrations of test compound, sodium nitroprusside 0.3 ml was added. The test tubes were incubated at 25 ºC for 5 hr after which, 0.5 ml of Griess reagent was added. The absorbance of the chromophore was read at 546 nm. The experiment was performed in triplicates.

Ferric reducing antioxidant power assay (FRAP ASSAY)

Total antioxidant activity was measured by ferric reducing antioxidant power (FRAP) assay of Benzie & Strain [9] and William *et al*[10].

RESULTS AND DISCUSSIONS

Phytochemical analysis

The presence of medicinally important molecules in a plant is indicative of its medicinal potential. *Turnera ulmifolia* was tested for the presence of alkaloids, flavonoids, anthraquinone, saponin, tannin, phlobotannin, terpenoids and cardiac glycosides. Alkaloids, anthraquinones, and cardiac glycosides were absent in all the samples (Table 1). Flavonoids were present in hexane,

ethyl acetate, and methanol extracts. Flavonoids are basically water soluble polyphenols so the presence of same in hexane extract was a bit contradictory but its presence in polar extracts like ethyl acetate and methanol indicates antioxidant, anti inflammatory and anti cancer potential of the plant as flavonoids have a proven role in all the above medicinal values[11]. Another important molecule terpenoid was present in all the extracts of the plant, is also a pointer towards the medicinal potential of the leaf extracts. The study was in confirmation with an earlier study in which terpenoids was reported in *Turnera diffusa*[12]. Saponin was found only in methanol extract whereas tannin and phlobotanin were present in ethyl acetate and methanol extracts. Thus, it could be assumed that polar extracts showed more medicinal potential in comparison to non-polar extracts.

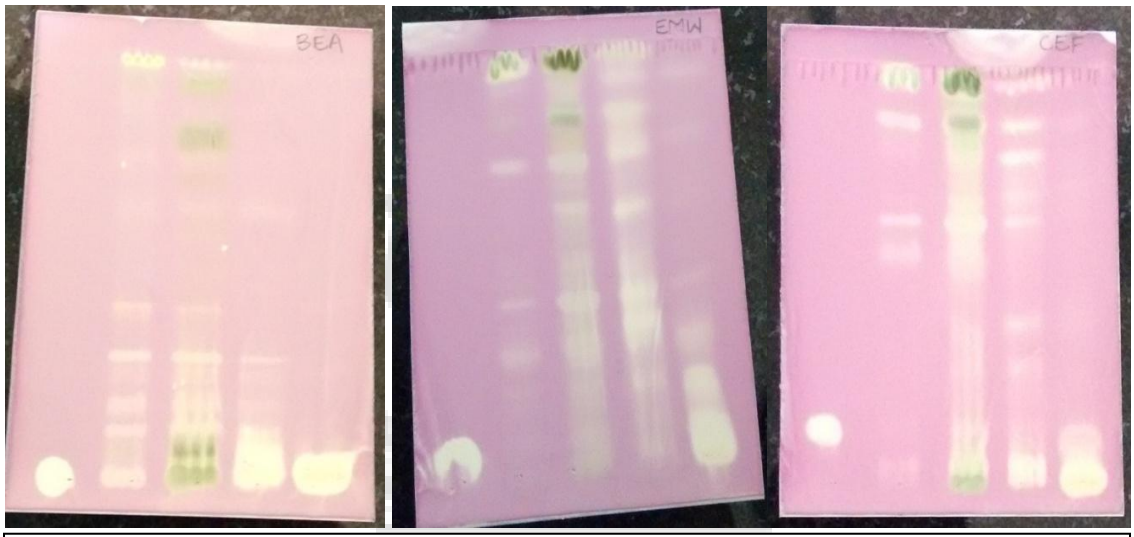
Table: 1 phytochemical analysis of fresh and solvent extracts of *Turnera ulmifolia* leaves

Phytoconstituents	Fresh leaves	Hexane	Chloroform	Ethyl acetate	Methanol
Alkaloid	-	-	-	-	-
Flavonoids	-	+	-	+	+
Anthraquinone	-	-	-	-	-
Saponins	-	-	-	-	+
Tannin	+	-	-	+	+
Phlobatannin	+	-	-	+	+
Terpenoids	+	+	+	+	+
Cardiac glycosides	-	-	-	-	-

Antioxidant activity

Qualitative antioxidant screening

TLC based antioxidant results of leaf extracts were promising as all the extracts showed a number of antioxidant bands as can be seen in Fig 1. Three solvents were used for chromatographic separation and all of them showed good separation with a maximum number of antioxidant bands in ethyl acetate fraction. Qualitative assay indicated a number of probable antioxidant molecules in an extract.



1= Ascorbic acid 2= Hexane ext. 3= Chloroform ext. 4= ethyl acetate ext.

5= Methanol extract.

Quantitative antioxidant study

DPPH free radical scavenging assay

The antioxidant activity of different extracts of *Turnera ulmifolia* was analyzed with DPPH, a stable free radical. As DPPH picks up one electron in the presence of free radical scavenger, the absorption decrease, and the resulting discoloration was related to the number of electrons

gained. Ethyl acetate extract of *Turnera ulmifolia* exerted an inhibition of 77.34% and that of ascorbic acid was 92.72% at 500µg/ml. The IC50 of ethyl acetate extract was 251.767µg/ml while that of ascorbic acid was 16.218µg/ml. Although activity of the extract was lower but it is a mixture of a number of molecules which can act synergistically as well as mask the effect of another molecule. Thus, ethyl acetate extracts need to be further explored for isolating future antioxidant principle. Remaining extracts showed negligible antioxidant activity (Fig 2)

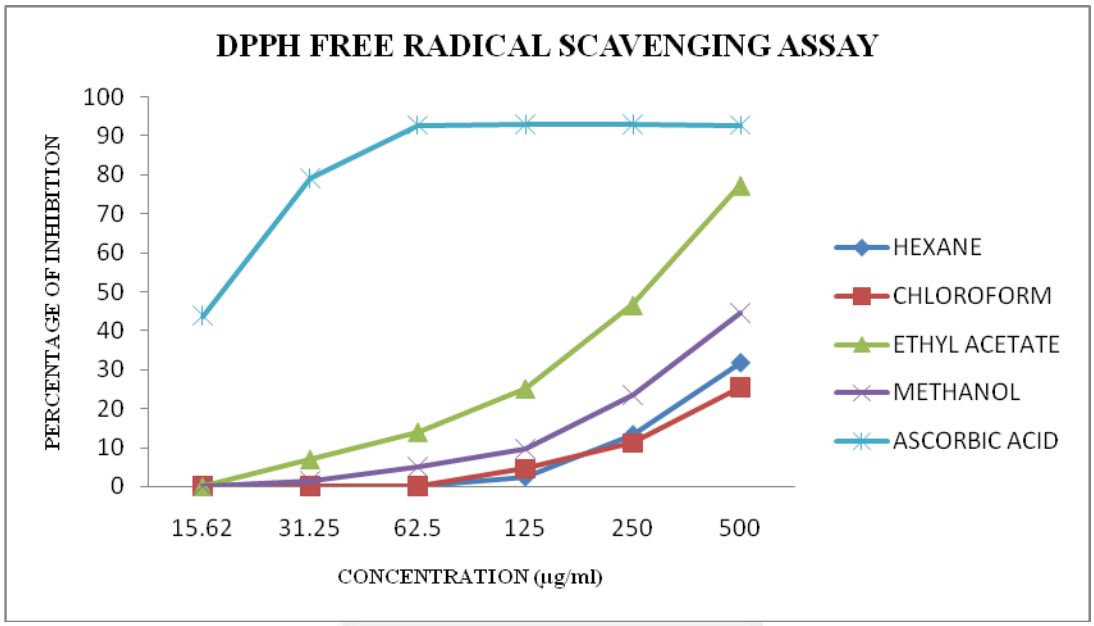


Fig 2:- DPPH FREE RADICAL SCAVENGING ASSAY

Ferric reducing antioxidant power assay

T.ulmifolia leaf extracts showed concentration-dependent reducing power. All the extracts showed only mild activity suggesting that the antioxidant molecules which were observed in the quantitative tests do not act synergistically instead mask each others effect.

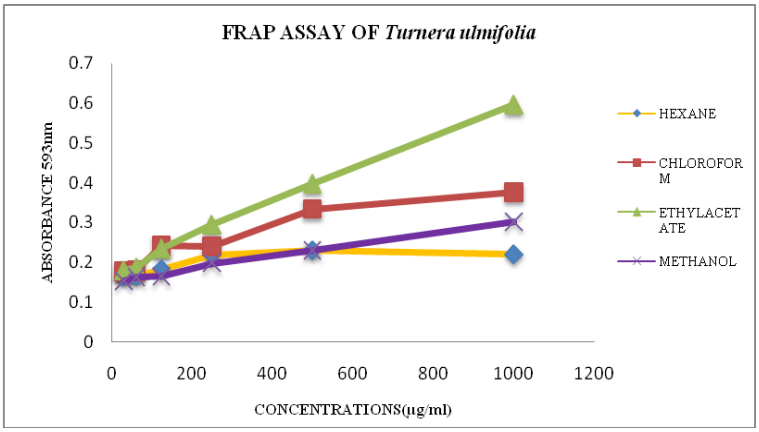


Fig 3: FRAP assay of leaf extracts of *Turnera ulmifolia*

Nitric oxide radical scavenging assay

Nitric oxide (NO.) is an important chemical mediator generated by endothelial cells, macrophages, neurons, etc. and involved in the regulation of various physiological processes. Excess concentration of nitric oxide is associated with several diseases such as vascular collapse, various carcinoma, and ulcerative colitis. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions (ONOO-), which act as free radicals [13]. In the present study, the plant extracts compete with oxygen to react with nitric oxide and thus inhibits generation of the anions. As can be seen from Fig 4, none of the extracts was more active than standard quercetin and all of them showed dose-dependent mild activity.

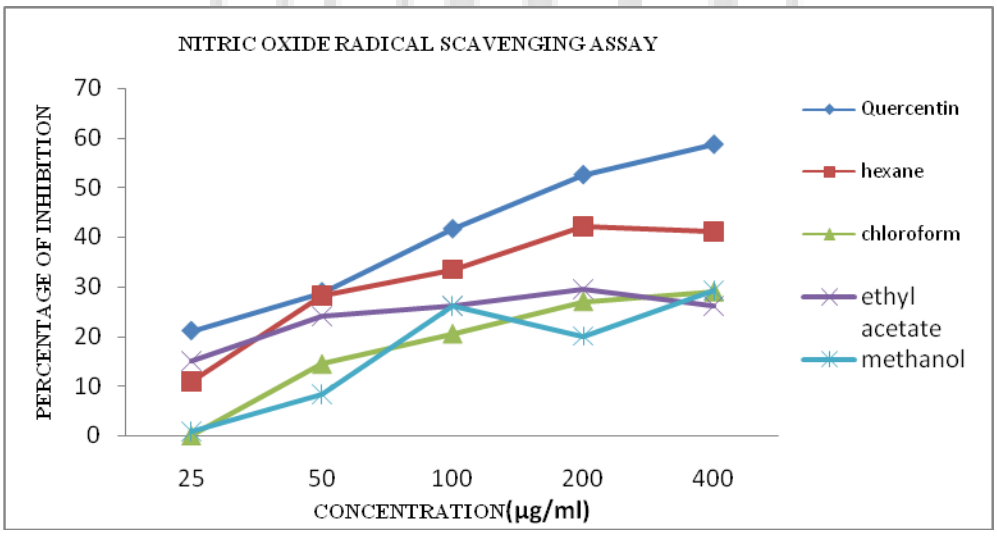
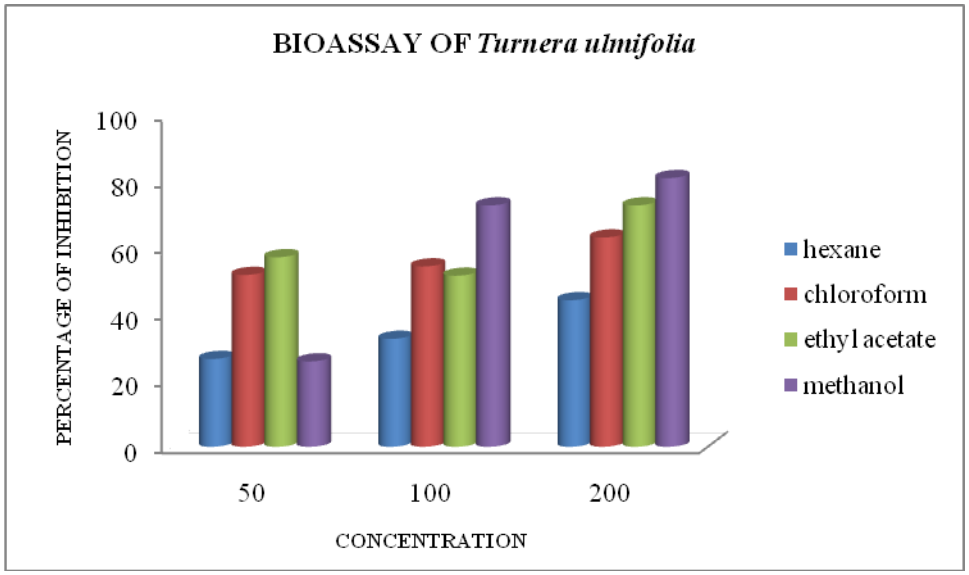


Fig 4: Nitric oxide radical scavenging assay

Cytotoxic activity of leaf extracts of *Turnera ulmifolia*

Artemia salina is one of the convenient organisms for toxicity tests in vitro due to its robust nature and easy maintenance in the laboratory condition. The bioassay study with Artemia larvae was carried out as short-term toxicity test and only on freshly hatched nauplii. The dose-dependent mortality was obtained in most of the extracts. The methanol extract of *T. ulmifolia* showed maximum cytotoxicity i.e. 80.94% at 200µg/ml followed by ethyl acetate extract which showed 75% cytotoxic activity. This study is in confirmation with the earlier study in which methanolic extracts has shown cytotoxic potential against breast cancer cells[14].



Overall it can be concluded that leaf extracts of *Turnera ulmifolia* possessed a number of medicinally important phytomolecules like flavonoids, tannin, and saponins. Although antioxidant properties of the plant were found to be mild in comparison with the standard compounds ascorbic acid and quercetin yet a number of antioxidant molecules were observed in the extracts in a quantitative assay. It seems if isolated could be potential antioxidant principles. Cytotoxic activity of methanol extracts was significant and warrants further exploration for the isolation of cytotoxic principles.

Acknowledgements

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