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Antioxidant Activity of the Isolated Compounds, Methanolic and Hexane Extracts of *Toona ciliata* Leaves

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ABSTRACT

Medicinal plants are the backbone in the treatment of the traditional medicine. *Toona ciliata has* been used for various ailments. *Toona ciliata* serves as a source of flavanoids, terpenoids and many phenolic compounds. Eight compounds were isolated from hexane and methanol extracts of *Toona ciliata.* The present study was undertaken to find out the antioxidant activity of the isolated compounds, hexane, methanol extracts of the *Toona ciliata* leaves. The antioxidant activity of the samples were determined using 2,2 – Diphenyl – 1 – Picrylhydrazyl (DPPH) free radical scavenging assay, hydrogen peroxide scavenging assay and reducing power assay. Further characterisation and structural elucidation of the isolated compounds has to be done with IR, NMR, Mass spectrometry.

Keywords : *Toona ciliata, 2,2 – Diphenyl – 1 – Picrylhydrazyl, Hydrogen peroxide, Reducing power*

I. INTRODUCTION

Free radicals are generated inside the human body as a product of normal metabolism. The reactive oxygen species (ROS) generated will attack the biomolecules and causes tissue damage[1-3].Antioxidants present in our body detoxify them and protect the tissues from oxidative damage[4-6]. But when the antioxidant defence is insufficient, the oxidative damage will occur which is implicated in many diseases like cardiovascular disorders, stroke, cancer, diabetes mellitus, cirrhosis, atherosclerosis and arthritis[7-10]. Studies have proven that the use of antioxidants like Vitamin C reduces the risk of heart diseases and cancer[11]. Risk of chronic diseases and disease progression can be reduced by boosting up the body's antioxidant defense mechanism or by supplementing with dietary antioxidants[12]. Plants contain many antioxidants like terpenoids, flavanoids and phenolic compounds which act by scavenging the free radicals and thereby reducing the tissue injury to the tissue[13].There are many synthetic antioxidants available, but they produce many unwanted effects in the human[14, 15]. Recently the search for the medicinal plants containing antioxidants has been increased. Thorough phytochemical and pharmacological screening of the plants can lead to the development of effective antioxidants[16, 17]. The leaves, stems and seeds of the *Toona ciliata* have been used traditionally for various ailments like diarrhoea, ulcer, leprosy, headache and cancer [18]. The aim of the present work is to determine the antioxidant activity of the isolated compounds hexane and methanolic extracts of *Toona ciliata* leaves.

II. MATERIALS AND METHOD

2.1 Collection of Plant Material:

Toona ciliata leaves were collected from the forest area of Coimbatore, Tamilnadu. The leaves were shade dried, homogenised to fine powder using a blender.

2.2 Extraction

The components present in *Toona ciliata* were extracted by cold maceration method. The powder was extracted successively in hexane and methanol. To 100g of powder 300ml of solvent was added and left for 24 hours. After 24 hours the extract was filtered over Whattman filter paper No.1.The process was repeated twice and all the collected extracts were pooled and evaporated to dryness under reduced pressure using rotary evaporator.

2.3 Isolation of Compounds:

The dried residues of both extracts were reconstituted with the respective solvents. Both the extracts were tested by Thin Layer Chromatography with various organic solvents. Finally Hexane:Ethyl Acetate was decided as the solvent system for both hexane and methanol extracts. There was clear separation of 4 compounds from both the extracts. The compounds obtained from hexane and methanol extracts are named as H1, H2, H3, H4 and M1, M2, M3, M4 respectively.

2.4 Determination of Antioxidant activity

2.4.1 Radical Scavenging Activity (DPPH Assay):

The antioxidant potential of any compound is measured on the basis of its ability to scavenge stable 2, 2 – Diphenyl – 1 – Picrylhydrazyl (DPPH) free radical [19]. DPPH contains an odd electron in its structure and is used for detection of radical scavenging activity of compounds[20]. The free radical scavenging activity of the extracts was measured using the slightly modified method of Molan *et al* [3]. A solution of 0.2 mM 2,2 – Diphenyl- 1- Picryl Hydrazyl was prepared in methanol. To 250µl of DPPH, 10µl of each sample (100µg/ml) were added in a 96- well microplate. The plate was incubated at room temperature in dark for 30 minutes. Absorbance was

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measured at 517nm using ELISA reader and all the determinations were performed in triplicate. Ascorbic acid was used as a positive control. Methanol with DPPH was used as blank. The percentage scavenging activity was calculated using the formula:

Radical scavenging activity (%)

$$= [(A_b - A_s) / A_b] * 100$$

A_b = Absorbance of the blank

A_s = Absorbance of the sample

2.4.2 Hydrogen Peroxide scavenging activity

The ability of the extracts to scavenge hydrogen peroxide was determined using the method of Ruch *et al* [21]. 40mM hydrogen peroxide was prepared in phosphate buffer (pH – 7.4). The samples (100µl/ml) were added to 0.6ml 40 mM hydrogen peroxide solutions. Absorbance of hydrogen peroxide was measured at 230nm 10 minutes later against a blank solution (Phosphate buffer without hydrogen peroxide). The hydrogen peroxide scavenging percentage was calculated according to the formula:

$$H_2O_2 \text{ scavenged } (\%) = A_c - A_s / A_c \times 100$$

A_c = Absorbance of the control

A_s = Absorbance in the presence of sample of *Toona ciliata* and standards

2.4.3 Reducing Power Assay

The reducing power assay of the samples was determined by using the method of Ferreira *et al* [22]. The samples (100µg/ml) were mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6) and 2.5ml of 1 % potassium ferricyanide. The mixture was incubated for 20 minutes at 50º C. 2.5 ml of 10% Trichloroacetic acid was added and the mixture was centrifuged at 1000rpm for 10 minutes. 2.5ml of distilled water and 0.5ml of 0.1% ferric chloride solution was added to 2.5ml of the supernatant. Absorbance was measured at 700nm after standing for 10 minutes. Higher the absorbance of the mixture higher the reducing power.

III. RESULTS AND DISCUSSION

The antioxidants produce their effects by reacting with ROS, quenching them or by chelating the catalytic metal ions. Many assays are used to determine the antioxidant activity but the widely used methods are those that involve free radical generation which are then neutralised by compounds having antioxidant property [13].

3.1 DPPH Free radical scavenging activity

DPPH is widely used and the most accepted chemical to test the ability of compounds to act as free radical scavengers or hydrogen donors. DPPH is a stable free radical at room temperature [23]. It accepts an electron or hydrogen atom and becomes a stable diamagnetic molecule. DPPH reduction capacity induced by the antioxidants was determined by decrease in the absorbance at 517nm. The decrease in DPPH absorbance is due to the scavenging of the free radical by hydrogen donation [15]. The percentage of DPPH scavenging activity of the samples were in the order of ME > M4> M2 >M3 > M1 > HE > H4> H1>H2>H3(Table 1) All the samples tested have shown good percentage of the free radical scavenging activity. Among them the crude methanol extract has shown maximal percentage inhibition 67.87 ± 0.24. The percentage inhibition of ascorbic acid which was used as the standard drug was 98.09 ± 0.05. All the tested samples shows antioxidant activity but less than the standard. Results of this assay suggest that the flavonoids, terpenoids and phenolic compounds present in the *Toona ciliata* may be responsible for this scavenging activity.

3.2 Hydrogen Peroxide scavenging activity

There are many reactive oxygen species, among which H_2O_2 is the most reactive radical generating molecule during metabolism. H2O2 degrades into oxygen and water, producing hydroxyl radicals leading to lipid peroxidation which produces DNA damage. The percentage of hydrogen peroxide scavenging activity of the samples were in the order of HE > M4> M2 >ME > M1 > M3 > H1> H2>H4> H3 (Table 1). Among them the crude hexane extract has shown maximal percentage inhibition 82.39 ± 9.47 which is more than the percentage inhibition of ascorbic acid 75.50 ± 2.95 which was used as the standard drug. Hexane extract efficiently scavenges H_2O_2 which may be due to the presence of flavonoids and phenolic compounds.

3.3 Reducing power assay

The reducing power assay is used to estimate the ability of any antioxidant to reduce Fe^{3+} to Fe^{2+} by donating an electron [24]. The compounds having antioxidant activity reduce the Fe^{3+} to Fe^{2+} thereby, changing the colour of the solution into various shades from green to blue, depending on the reducing power of the compounds. The absorbance of the samples were in the order of M4> H2 >M3 > H4 > M2 > M1> ME >H3 >H1 >HE (Figure 1). Among them M4 has shown maximal absorbance of 0.440 ± 0.001 which is equivalent to the absorbance of ascorbic acid 0.461 ± 0.003 which was used as the standard drug. The reducing power of the compounds is probably due their hydrogen donating ability.

IV. CONCLUSION

Many of the medicinal plants in India are common and growing as weeds in wild conditions. From the results obtained, it can be concluded that *Toona ciliata* extracts and the compounds isolated from them have promising potential to serve as a source of natural antioxidants. Characterisation and structure elucidation of the isolated compounds of the extracts may lead to the development of drugs leading to commercialization for large scale use.

Table 1: DPPH and Hydrogen Peroxide scavenging activity of the isolated compounds, hexane and methanolic extracts of *Toona ciliata* leaves

Samples (100µg/ml)	DPPH scavenging activity %	Hydrogen Peroxide scavenging activity %
HE	21.39 ± 1.10	82.39 ± 9.47
H1	17.13 ± 0.005	40.29 ± 3.19
H2	17.13 ± 0.06	34.90 ± 1.04
H3	16.67 ± 0.03	26.49 ± 1.31
H4	18.59 ± 0.01	33.90 ± 1.17
ME	67.87 ± 0.24	55.69 ± 1.04
M1	40.28 ± 0.04	51.84 ± 1.23
M2	62.67 ± 0.21	57.39 ± 1.31
M3	52.41 ± 0.01	45.85 ± 1.97
M4	64.85 ± 0.0058	62.95 ± 3.31
Ascorbic acid	98.09 ± 0.05	75.50 ± 2.95

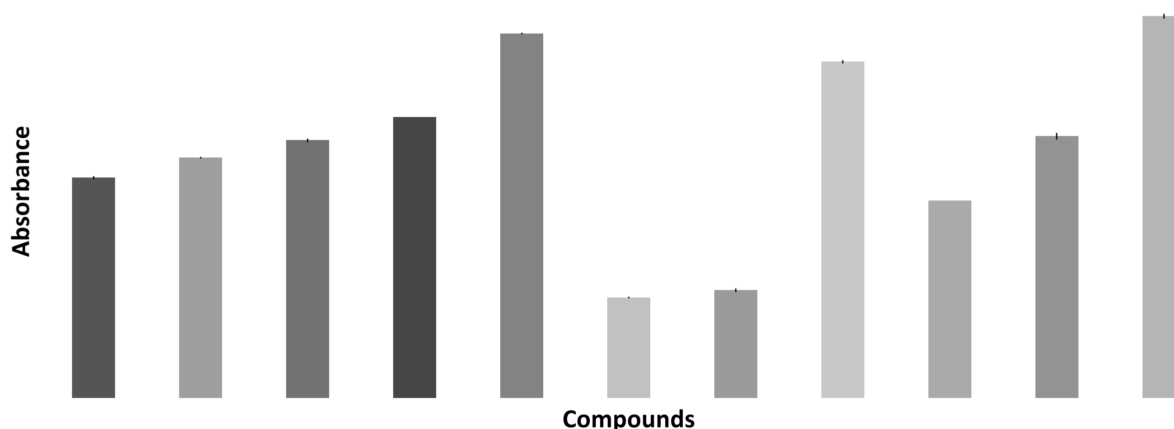

Figure.1 Reducing power assay of the isolated compounds, hexane and methanolic extracts of Toona ciliata leaves

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