Antioxidant activity of medicinal plants in *Thespesia populnea* L. and *Abutilon indicu*m L. by various methods

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ABSTRACT

The determination of the antioxidant activity of selected medicinal plants namely *Thespesia populnea* and *Abutilon indicu*m L. leaves has been studied using different solvents of aqueous, diethyl ether and methanol extracts were used. *Invitro* antioxidant potential of leaves of *T.populnea* leaf and *A. indicum* L. with three different methods like hydrogen peroxide scavenging (H_2O_2) assay, reducing power assay and Thiobarbataric acid was evaluated. The three solvent extracts with different concentration of 0.2, 0.4, 0.6, 0.8 and 1.0 percentage were prepared. Determination of antioxidant assay of different concentration of plant extract for more suitable in Thiobarbataric acid activity than the other methods. The aqueous, methanol and diethyl ether extract of *T. populnea* (1%) has excellent percentage of activity when compared with low concentration of plant extract whereas *A.indicum* leaves with aqueous, methanol and diethyl ether extract also moderate percentage of activity from reducing power assay were observed. In comparison, the antioxidant activity of maximum percentage of activity in *Abutilon indicum* leaves when compared with *Thespesia populnea* . These observations confirm that methanolic extract of *Thespesia populnea* and *Abutilon indicu*m leaves have different polyphenolic constituents and its importance in antioxidant activity.

Key words: Medicinal plants, solvents

INTRODUCTION

Medicinal plants have undoubtedly been considered by human beings since ancient times. It can be said that before the history and since the early humans recognized and exploited the plants around them for use as fuel, clothing, shelter and food, they became aware of their properties more or less.

The medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free-radicals (Kahkonen *et al.,* 1999). They are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers. Antioxidants from plant materials terminate the action of free radicals thereby protecting the body from various diseases (Lai and Chou 2001). There is a growing interest all over the world for discovering the untapped reservoir of

medicinal plants. Hence, the present study was aimed at measuring the relative content of antioxidant capacities of important medicinal plants.

Antioxidants are defined as a substance that even in small amounts is capable of preventing or delaying the oxidation of easily oxidizable materials. Antioxidant are also defined as a substance which are capable of inhibiting a specific oxidising enzymes or a substance that reacts with oxidizing agents prior to causing damage to other molecules or a substance that sequesters metal ions or even a substance capable of repairing system such as iron trans porting protein (Brewer 2011).

MATERIALS AND METHODS

Collection of plant materials

Healthy plants of *Thespesia populnea* L. and *Abutilon indicu*m L. were collected from Gopal nagar, Thanjavur, Tamilnadu, India. The leaf materials were cleaned and free from dirt particles and shade dried.

Preparation of plant extracts

Soxhlet method used for extraction of crude materials One gram of powder leaves blended with 50 ml of different solvents separately (aqueous, methanol and diethyl ether) for different periods with agitation at room temperature. After the extracts were allowed to filtration by using a 0.45 Millipore filter paper. The plant extracts concentrated using a rotary evaporator at 40°C under reduced pressure. Finally the extracts were allowed to weigh and store at -20°C till their usage in the different tests.

Antioxidant activity

Hydrogen Peroxide Scavenging assay (Ruch *et al.*, 1989).

The ability of the *Thespesia populnea* and *Abutilon indicu*m extracts to scavenging hydrogen peroxide was determined according the method of solution of hydrogen peroxide (40mm) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/ml) in distilled water were added to a hydrogen peroxide solution (0.6 ml, 40ml). Absorbance of hydrogen peroxide scavenging assay at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of antioxidant activity.

$$\% \text{ Scavenged } [H_2O_2] = [(AC - AS)/AC] \times 100$$

Reducing power assay (Oyaizu, 1986)

The reducing power assay with aqueous extract was determined according to this method. One ml of the leaf extract containing (0.2 – 1.0 microgram) in 1ml of the deionized water mixed with 2.5ml of

phosphate buffer (0.2M, pH 6.6) and 2.5ml potassium ferrocyanide (1%). The mixture was incubated at 50°C for 20 minutes. 2.5ml of TCA (10%) and centrifuged at 3000 rpm. The upper layer of the solution was mixed with 2.5ml distilled water and $FeCl_3$ (0.5ml, 0.1%). The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated the higher reducing power. The absorbance compared with the standard ascorbic acid (concentration 20 µg).

The percent increase in reducing power assay was calculated using the following equation

$$\text{Increase in reducing power (\%)} = \frac{\text{A test - A std}}{\text{A}} \times 100$$

Thiobarbitaric Acid (TBA) Method (Sawarka *et al*., 2009)

TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100ºC), melonaldehyde binds with TBA to form a red complex that can measured at 532 nm and 2 ml of 20% Trichloroacetic acid and 2 ml of 0.67%TBA solutions were added to 2 ml of the mixtures containing the sample (0.2 – 0.8 microgram) prepared in the FTC (Ferric thiocyanate) method. The percentage of antioxidant activity was calculated by following formula for TBA.

$$\text{(\%) Percentage of activity} = \frac{\text{Absorbance of (Control}-\text{Test)}}{\text{Absorbance Control}} \times 10$$

RESULT AND DISCUSSION

In the present investigation suggested that the determination of effect of antioxidant activity of medicinal plants like *T.populnea* and *A.indicum* was carried out by three methods. Among the three methods, the Thiobarbataric acid assay showed excellent antioxidant activity from *T.populnea* with aqueous extract when compared with other methods of hydrogen peroxide scavenging assay and reducing power assay whereas the aqueous solvent extract of *A.indicum* plant has enomorus free radical scavenging activity from reducing power assay was 24.5±3.08,35.4±1.11,23.7±3.14,19.2±8.13and 48.1±4.16 percentage activity observed with respective plant *A.indicum* concentration of 0.2,0.4,0.6,0.8 and 1.0 percentage treated respectively. The maximum percentage of antioxidant activity was observed due to the properties of phytochemical activity (Table – 1)

Screening of compounds which scavenge the free radicals, could lead to promising compounds. Most of the antioxidants used in therapy are derived from natural sources. About 28 % drugs approved by the FDA between 1981 and 2002 are either natural products or chemicals derived from them (Clardy and Walsh

2004). Many synthetic antioxidant components have shown toxic and/or mutagenic effects. The attention has been given to naturally occurring antioxidants. Therefore, identification of effective antioxidants and free radical scavengers from plant origin is an ideal strategy for new drug development. Hence, present study was designed to explore the antioxidant potential and free radical scavenging activity of *T. populnea* and *A. indicum* leaves by various methods.

According to the methanolc extract of *T.populnea* with different concentration of 0.2,0.4,0.6,0.8, and 1.0 % has developed ionic stability to promote the activity was 29.3±2.42, 22.1±0.71, 25.6±1.40, 12.4±3.94 and 35.2±1.66% observed from Thiobarbataric acid than the other methods whereas *A.indicum* plant extract free radical scavenging activity of methanolic extract has been investigated by reducing power assay recorded respectively (Table - 2).

Antioxidant activity, total phenolic and flavonoid content of leaf, stem and bark extracts of *B. aristata* and *B. thomsoniana* was determined. Considerable amount of variation was observed among the samples regarding antioxidant ability, total phenolic and flavonoid contents. Among the tested samples, leaf extract of *B. thomsoniana* exhibited highest antioxidant activity. However, the increasing concentration there was decrease in radical scavenging activity (RSA). It is like that 0.625 mg/g concentration of the sample is quite enough to quench the available DPPH radicals. The DPPH radicals would have contributed in decreasing the RSA of *B. thomsoniana* leaf extract on increasing the sample concentration. (Lok Ranjan Bhatt *et al*.,2018).

The diethyl ether solvent extract of *T.populnea* with different concentration like 0.2,0.4,0.6,0.8 and 1.0 % of leaf extract showed maximum activity of free radical scavenging assay was 17.6±06.4,19.8±07.6±,15.6±0.82,13.6±12.7,and 24.8±11.6% antioxidant activity though that delays or inhibits oxidative damage to a target molecules repressed respectively whereas the reducing power assay of *A.indicum* leaf extract may serve as a significant indicator of its potential antioxidant activity was 14.0±0.10, 10.4±0.21, 17.8±0.17, 11.1±0.09 and 22.5±0.15% of activity from respective concentration of plant extract. The presence of reductions which have been showed to free radical chain by donating a hydrogen atom. This may be serried as significant indicator of its potential antioxidant activity. Hence this study presumed that the methanol extracts of leaf part of *T. populnea* may have high amount of reductions and hence the antioxidant property. Therefore, these plant species may be attempted to derive the antioxidant properties were observed.

DPPH radical scavenging activity, ABTS radical scavenging activity, hydrogen peroxide radical scavenging activity and ferric reducing antioxidant power method. The expressed as percentage inhibition and IC50 (where 50 % concentration of the extract scavenged free radical). The lower the IC50 (µg/ml) value the higher the percentage inhibition on free radicals. Antioxidant activity determined using DPPH radical scavenging activity revealed that *S. occidentalis* extract (IC50: 41.80 µg/ml) scavenged free radical activity compared to other methanol extracts and gallic acid (IC50: 48.77 µg/ml).

Furthermore *S. occidentalis* extract (IC50:69.19 µg/ml) exhibited strong activity compared to other extracts. L-ascorbic acid (IC50:39.80 µg/ml) exhibited strong antioxidant activity using Ferric reducing antioxidant power (Thagriki Dluya *et al.*, 2017).The free radicals are reaction oxygen and nitrogen species which are generated by various physiological processes in the body.

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Table 1: Determination of antioxidant activity of *Thespesia populnea* and *Abutilon indicum* leaves with aqueous extract by *invitro* method

Different concentration (%)	*Thespesia populnea*			*Abutilon indicum*		
	Hydrogen peroxide scavenging (H_2O_2) assay	Reducing power assay	Thiobarbataric acid	Hydrogen peroxide scavenging (H_2O_2) assay	Reducing power assay	Thiobarbataric acid
	(%) activity	(%) activity	(%) activity	(%) activity	(%) activity	(%) activity
0.2	11.3±3.20	16.4±4.74	29.4±44.2	6.18±1.06	24.5±3.08	21.3±1.12
0.4	10.0±1.73	13.6±7.96	22.7±67.1	2.22±1.07	35.4±1.11	13.1±3.16
0.6	12.3±0.79	25.3±8.55	25.9±42.3	8.23±5.07	23.7±3.14	24.5±1.10
0.8	16.7±4.82	19.4±1.25	12.0±97.7	3.32±3.10	19.2±8.13	20.1±4.21
1.0	11.9±3.32	20.1±1.43	35.1±26.5	9.43±7.14	48.1±4.16	14.4±8.13

Standard deviation ±error

Table 2: Determination of antioxidant activity of *Thespesia populnea* and *Abutilon indicum* leaves with methanolic extract by *invitro* method

Different concentration (%)	*Thespesia populnea*			*Abutilon indicum*		
	Hydrogen peroxide scavenging (H_2O_2) assay	Reducing power assay	Thiobarbataric acid	Hydrogen peroxide scavenging (H_2O_2) assay	Reducing power assay	Thiobarbataric acid
	(%) activity	(%) activity	(%) activity	(%) activity	(%) activity	(%) activity
0.2	3.10±0.42	5.16±3.44	29.3±2.42	23.4±6.21	17.2±14.0	11±0.12
0.4	6.21±0.87	2.13±2.76	22.1±0.71	38.2±3.10	14.1±15.1	33±0.15
0.6	1.22±0.67	1.25±1.85	25.6±1.40	42.4±2.07	18.4±13.3	35±0.21
0.8	3.12±0.18	1.30±3.15	12.4±3.94	27.2±1.13	32.1±11.0	24±0.20
1.0	7.32±0.33	4.20±4.23	35.2±1.66	19.3±0.14	40.2±16.2	16±0.19

Standard deviation ±error

Table 3: Determination of antioxidant activity of *Thespesia populnea* and *Abutilon indicum* leaves with diethyl ether extract by *invitro* method

Different concentration (%)	*Thespesia populnea*			*Abutilon indicum*		
	Hydrogen peroxide scavenging (H_2O_2) assay	Reducing power assay	Thiobarbataric acid	Hydrogen peroxide scavenging (H_2O_2) assay	Reducing power assay	Thiobarbataric acid
	(%) activity	(%) activity	(%) activity	(%) activity	(%) activity	(%) activity
0.2	11.7±14.2	08.2±03.4	17.6±06.4	12.4±0.06	14.0±0.10	12.4±5.13
0.4	16.2±01.7	13.4±07.6	19.8±07.6	11.2±0.03	10.4±0.21	11.6±2.17
0.6	10.7±15.9	15.6±09.5	15.6±08.2	13.1±0.09	17.8±0.17	15.3±7.06
0.8	25.6±10.8	11.8±07.2	13.6±12.7	18.5±0.11	11.1±0.09	10.2±4.12
1.0	12.9±13.3	16.3±11.3	24.8±11.6	12.7±0.12	22.5±0.15	15.6±1.18

Standard deviation ±error

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