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Annals of Biological Research, 2021, 13 (3):129-133
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0976-1233
CODEN (USA): ABRNBW

Antioxidant Assay of Some Local Medicinal Plants

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Received: *07-Mar-2022, Manuscript No. ABR-22-54048;* ***Editor assigned:*** *09-Mar-2022, PreQC No. ABR-22-54048 (PQ);* ***Reviewed:*** *21-Mar-2022, QC No ABR-22-54048;* ***Revised:*** *25-Mar-2022, Manuscript No ABR-22-54048(R)*
Published: *05-Apr-2022. Doi: 10.4172/ 0976-1233.005*

ABSTRACT

Plants are the basic source of knowledge of modern medicine. The increased worldwide interest in medicinal plants reflects the value of natural products in health care. The relatively low incidence of adverse reactions to plant preparations compared to modern pharmaceuticals to consider plant medicines as alternatives to synthetic drugs. People are becoming increasingly interested in medicinal plants because of their good therapeutic performance and low toxicity. Some plant extracts are believed to have strong antioxidant effects. Increased use of plant extracts in food, cosmetics and pharmaceutical industries suggest that in order to find the active compound a systematic study of medicinal plants is very important. Many Indian plants are considered as potential source of antioxidant compounds. Hence investigations have been taken up for which four common medicinal plants have been selected i.e, Drumstick, Tamarind, Custard apple and Mint to study their potential antioxidant activity using crude ethanol, aqueous and chloroform extracts of the plants. Antioxidant assays such as superoxide radical scavenging, DPPH and FRAP analysis for the plant extracts by spectrophotometric methods using known standard oxidants as reference were performed. The study reveals that Tamarind has the highest antioxidant activity followed by Mint.

Keywords: Antioxidant, Standard antioxidants, Superoxide, Ethanolic

INTRODUCTION

A wide range of medicinal plant parts are used as raw drugs and they possess varied medicinal properties [1]. Although hundreds of plant species have been tested for antimicrobial properties, vast majority have not been adequately evaluated. The beneficial medicinal effects of plant material result from the combination of secondary products present in the plants. The combination of secondary products in a particular plant is often taxonomically distinct [2]. Plant secondary products have protective action in relation to abiotic stresses and the potential role of secondary products at cellular level as plant growth regulators, modulators of gene expression and in signal transduction. It is likely that their ecological function may have some bearing on potential medicinal effects for humans. Antioxidant based drugs/formulations for the prevention and treatment of complex diseases have appeared during the last three decades. This has attracted a great deal of research interest in natural antioxidants. Several herbs have been reported to exhibit antioxidant activity. Antioxidants prevent cancer and heart disease to boosting immune system and slowing the ageing process [3]. Many Indian medicinal plants are considered potential sources of antioxidant compounds. In some case their active constituents are known. There is an increased quest to obtain natural antioxidants with broad spectrum action. The rich diversity of Indian medicinal plants is yet to be scientifically evaluated for such properties. In this report, investigations were carried out with crude ethanolic, aqueous and chloroform extracts of four common plants for their potential antioxidant activity with Diphenyl Picryl Hydrazyl (DPPH), superoxide radical scavenging and FRAP methods. Antioxidant assays were performed for the plant extracts by using spectrophotometric methods using known standard antioxidants as reference.

MATERIALS AND METHODS

Plant leaves of *Annona squamosa*, *Moringa oleifera*, *Tamarindus indica* and *Mentha spicata* were collected from different areas of Visakhapatnam. The taxonomic identities of plants were confirmed by University of Agricultural Sciences, Banglore, Karnataka, India .The fresh plant materials were washed with tap water, air dried, homogenized to a fine powder and stored in air tight bottles. A glass soxhlet extraction apparatus was used for plant extracts. The test specimen consists of approximately 25 gms of milled sample. Depending on the solvent used the temperature and time of extraction varies.

Distilled water (85-95) ℃ as its boiling point is1000 ℃

Ethanol (70%) 65-72 ℃ as its boiling point is 70.10 ℃

Chloroform (70%) 50-58 ℃ as its boiling point is 63.5 ℃

Antioxidant activity of leaf extract

The plant materials along with three solvents (water, ethanol and chloroform) were obtained subjected to evaporation, so that any remaining solvent gets evaporated. And the samples were weighed in order to make concentrations of 100 µg, 250 µg, 500 µg and 1000 µg each of water, chloroform and ethanol samples. These samples were further stored in air tight bottles for use in antioxidant studies. DPPH (Diphenyl Picryl Hydrazyl radical scavenging assay: The DPPH assay was carried out as describedby [4]. 5.0 ml of DPPH solution in methanol was added to 50 µl of sample. After 30min of incubation period at room temperature, the absorbance was read against a blank containing sample and methanol at 517 nm. Butylated Hydroxy Toluene (BHT) and Rutins were used as positive controls. The percent inhibition of DPPH radical is calculated by the formula (A0 –A) x 100/A0, where A0 is absorbance of control and A is absorbance of test sample and IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Superoxide radical scavenging assay was carried by the method of Beauchamp and based on the reduction of Nitro Blue Tetrazolium (NBT) [5]. To 0.5 ml of the plant extract 1 ml of sodium carbonate, 0.4 ml of NBT and 0.2 ml of EDTA were added. The reaction was initiated by adding 0.4 ml of Hydroxylamine hydrochloride. The absorbance was recorded at 560 nm, the control was simultaneously run without plant extract. The units of SOD were expressed as amount of enzyme required for inhibiting the reduction of NBT by 50%. The specific activity was expressed in terms of units per milligram of protein.

Ferric Reducing or Antioxidant Power Assay (FRAP)

The total antioxidant power of the sample was assayed by the method of antioxidant power assay [6]. At low pH, reduction of ferric tripyridyl triazine complex to the ferrous form, which has an intense blue colour, can be monitored by measuring the change in absorption at 593 nm. The change in absorbance is directly related to the combined or total reducing power of the electron donating antioxidants present in the reaction mixture. Aqueous solutions of known Fe (II) concentration and freshly prepared aqueous solutions of pure antioxidant, such as Ascorbic acid were used for calibration of FRAP assay [7].

RESULTS AND DISCUSSION

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Hence DPPH is usually used as substrate to evaluate the antioxidant activity. The strength of the scavenging activity of methanol extract and standards on DPPH radical was higher in *Tamarindus indica* followed by *Mentha spicata*, *Annona squamosa* and *Moringa oleifera* with percentage of inhibitions of 87.280,47.188, 38.331 and 37.102 respectively (Table 1), whereas the scavenging ability standards were found to be BHT-15.1 and Ascorbic acid-68.0. These results indicate that *T.indica* has shown significant effect on scavenging free radicals than Ascorbic acid and BHT. *M.spicata, A.squamosa* and *M.oleifera* has shown better activity than BHT and less activity when compared to with Tamarind and Ascorbic acid. Free radical scavenging activity also increased with increasing concentration (Table 1).

Based on the data obtained from this study, ethanolic extracts of all the four plants are free radical scavengers as well as primary antioxidant that react with free radicals, which may limit free radical damage occurring in the human body. Super oxide anion is one of the most representative free radicals. In biochemical systems, superoxide radicals can be converted to hydrogen peroxide by the action of dismutase and the H_2O_2 can subsequently generate extremely

reactive hydroxy radicals in the presence of certain transition metal ions. Hydroxy radicals can attack DNA molecules to cause strand scission [8]. The inhibitory effects of ethanolic plant extracts of *Tamarindus indica*, *Mentha spicata*, *Annona squamosa* and *Moringa oleifera* were found to be 85.350, 90.146, 84.321 and 90.642 (Table 3). The total antioxidant power of plant extracts are determined and results are expressed as FRAP units in µM. A standard graph was constructed with ascorbic acid concentrations 100 µM, 250 µM, 500 µM and 1000 µM and the values were expressed in terms of FRAP units. They showed significant total antioxidant power with values of 1300, 780, 430 and 310 FRAP units in µM [9,10]. The results indicate that highest total antioxidant power was found in *Tamarindus indica* with 1300 FRAP units followed by *Moringa oleifera* with 780 FRAP units, *Mentha spicata* with 430 FRAP units and *Annona squamosa* with 310 FRAP units respectively (Table- 2).

DPPH scavenging assay showed that at 1000 µg concentration the ethanol extract showed maximum activity with *Tamarindus indica*. Super oxide radical scavenging assay of all the four plant extracts showed that *Moringa oleifera* has highest scavenging activity followed by *Mentha spicata*. Results of total antioxidant assay reveal that Tamarind shows highest total antioxidant power followed by drumstick, mint and custard apple (Figures 1- 3).

Table 1: Diphenyl picryl hydrazyl radical scavenging assay (DPPH Assay)

% of Inhibition				
Name of the plant	**Conc. of plant extract**	**Aqueous**	**Chloroform**	**Ethanol**
Mentha spicata	100 µg	23.132 ± 0.15	26.910 ± 0.14	30.511 ± 0.14
	250 µg	27.683 ± 0.15	30.042 ± 0.18	37.013 ± 0.12
	500 µg	30.723 ± 0.18	33.887 ± 0.17	44.612 ± 0.14
	1000 µg	31.832 ± 0.12	33.818 ± 0.12	47.188 ± 0.14
Tamarindus indica	100 µg	25.927 ± 0.12	29.908 ± 0.16	37.066 ± 0.14
	250 µg	27.506 ± 0.12	30.438 ± 0.12	48.267 ± 0.18
	500 µg	27.859 ± 0.16	31.548 ± 0.14	69.909 ± 0.15
	1000 µg	28.215 ± 0.15	31.886 ± 0.12	87.208 ± 0.17
Moringa oleifera	100 µg	22.002 ± 0.12	27.046 ± 0.12	29.140 ± 0.15
	250 µg	25.225 ± 0.11	27.605 ± 0.18	31.324 ± 0.11
	500 µg	26.232 ± 0.13	28.571 ± 0.14	34.598 ± 0.13
	1000 µg	26.800 ± 0.11	32.011 ± 0.15	37.102 ± 0.12
Annona squamosa	100 µg	12.114 ± 0.12	16.922 ± 0.14	22.311 ± 0.11
	250 µg	17.233 ± 0.15	22.232 ± 0.15	27.013 ± 0.12
	500 µg	22.703 ± 0.11	23.887 ± 0.16	32.422 ± 0.15
	1000 µg	27.031 ± 0.12	32.018 ± 0.12	38.311 ± 0.12
Note: Values are means ± SD (n=2)				

Table 2: Total antioxidant assay-FRAP method

Name of the plant	**FRAP units in µM**
Mentha spicata	430 ± 0.04
Tamarindus indica	1300 ± 0.07
Moringa oleifera	780 ± 0.06
*Annona squamosa*l	310 ± 0.07
Note: Values are means ± SD (n=3)	

Table 3: Super oxide radical scavenging assay

		% of Inhibition		
Name of the plant	**Conc. of plant extract**	**Aqueous**	**Chloroform**	**Ethanol**
Mentha spicata	100 µg	26.190 ± 0.05	49.127 ± 0.04	51.790 ± 0.04
	250 µg	48.880 ± 0.07	69.579 ± 0.05	70.324 ± 0.06
	500 µg	64.705 ± 0.05	80.300 ± 0.08	81.533 ± 0.07
	1000 µg	78.571 ± 0.04	87.179 ± 0.05	90.146 ± 0.04
Tamarindus indica	100 µg	23.043 ± 0.06	44.165 ± 0.07	56.617 ± 0.02
	250 µg	31.034 ± 0.08	51.829 ± 0.04	66.244 ± 0.08
	500 µg	48.880 ± 0.03	68.533 ± 0.08	78.604 ± 0.06
	1000 µg	56.284 ± 0.07	78.545 ± 0.06	85.350 ± 0.05
Moringa oleifera	100 µg	11.195 ± 0.08	19.191 ± 0.02	39.487 ± 0.06
	250 µg	18.705 ± 0.02	38.461 ± 0.08	64.487 ± 0.02
	500 µg	20.261 ± 0.05	69.465 ± 0.04	83.462 ± 0.06
	1000 µg	24.421 ± 0.05	71.830 ± 0.06	90.642 ± 0.06
Annona squamosa	100 µg	13.120 ± 0.01	22.027 ± 0.02	38.543 ± 0.03
	250 µg	32.680 ± 0.07	36.324 ± 0.04	58.234 ± 0.08
	500 µg	45.445 ± 0.03	69.230 ± 0.08	75.635 ± 0.07
	1000 µg	62.471 ± 0.04	78.321 ± 0.07	84.231 ± 0.05
Note: Values are means ± SD (n=2)				

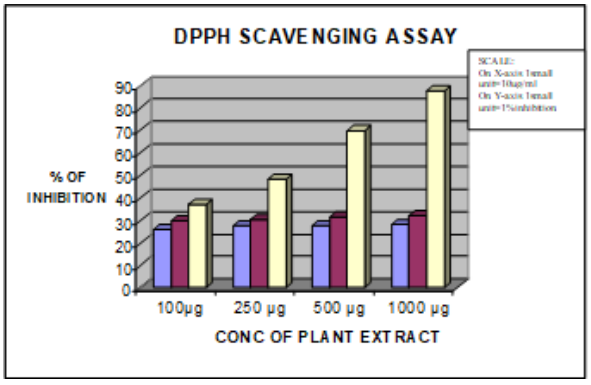

Figure 1: DPPH assay of Tamarind Plant, Note (▯) Aqueous; (▮)Chloroform; (▯) Ethanol

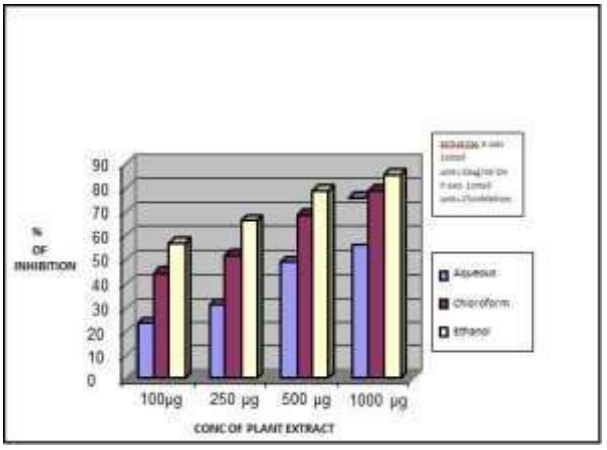

Figure 2: Superoxide Radical Scavenging Assay of Tamarind Plant, Note (▯) Aqueous; (▮)Chloroform; (▯) Ethanol

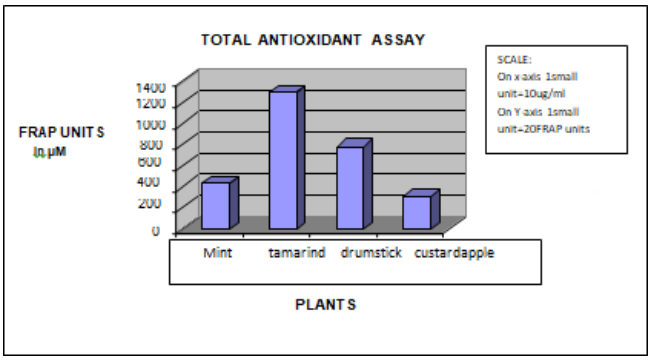

Figure 3: Total Antioxidant Assay, Note (■) Antioxidant activity

CONCLUSION

The antioxidant activity of all the four plants *Annona squamosa*, *Moringa oleifera*, *Tamarindus indica* and *Mentha spicata* as determined by several methods, has documented that these common plants are potential sources of antioxidant compounds which can be explored further to evaluate their beneficial properties as they are known to provide a layer of protection for cells and tissues. The yield of these compounds in cell cultures is though lower than in whole plants, it can be substantially increased by manipulating physiological and biochemical conditions.

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