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Nilesh S Chavan
Assistant Professor, MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Prashant B Kardile
Assistant Professor, (Plant Physiology) Department of Agricultural Botany, Dadasaheb Patil College of Agriculture, Dahegaon, Tal- Vaijapur, District- Auranagabad, Maharashtra, India

Kshama G Deshpande
Assistant Professor, MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Niraj M Kotgire
UG student (B.Sc. Agril. Biotechnology) MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Correspondence
Nilesh S Chavan
Assistant Professor, MGM College of Agricultural Biotechnology, Gandheli, Aurangabad, Maharashtra, India

Studies on superoxide anion radical scavenging activity, antioxidant activity, and reducing power of methanolic and aqueous extract of different medicinal plants

Nilesh S Chavan, Prashant B Kardile, Kshama G Deshpande and Niraj M Kotgire

Abstract
Present investigation entitled Studies on superoxide anion radical scavenging activity, antioxidant activity, and reducing power of methanolic and aqueous extract of different medicinal plants was carried out during December 2016 - April 2017 in Department of Biochemistry and Molecular Biology, MGM College of Agricultural Biotechnology, Gandheli, Aurangabad. Experiment was laid out in Factorial Randomized Block Design (FRBD) with ten treatments of different medicinal plants (Amla, amsul, chinch-Imli, neem, gavti chahaa, pipal, umber, wad, Pudina, coriender) and three replications. The healthy disease free medicinal plants were selected. The antioxidant activity of the medicinal plants extract and the standard were assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical activity by modified method. The dilute working solutions of the test extract were prepared in methanol. Ascorbic acid was used as standard in 1-100µg/ml solution. 0.002% of DPPH was prepared in methanol. These solution mixtures kept in dark for 30 min and optical density was measured at 517nm using Cecil-Elect Spectrophotometer. Methanol (1ml) with DPPH solution (0.002%, 1ml) was used as a blank.

Keywords: Super oxide anion radical, scavenging activity, antioxidant, methanolic, aqueous extract

Introduction
Plant have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicines.Antioxidants are chemicals that can prevent or slow cell damage. An antioxidant is an actually not a substance; it's a behavior. Any compound that can donate electrons counteract free has antioxidant properties. Natural antioxidant are mainly found in fruits and vegetables. There are thousands of antioxidant compounds out there, but the most common dietary once are vitamins A, C and E. Beta carotene and lycopene antioxidant can also be produced artificially and consumed in supplement form (Katalinic *et al.* 2006) [5].

Antioxidants are molecules that inhibit the oxidation of other molecules and thus, prevent free radicals, which cause damage to cell. A lot of type surrounds a group of compounds found in food called antioxidants. They are thought as everything from disease fighters to memory protectors to the antidote. Different medicinal plants are taken to evaluate their antioxidants properties with the help of aqueous and methanolic extract. Medicinal plants are taken for checking their antioxidant properties are *Phyllanthus emblica* (Amla), *Garcinia indica* (Aamsul), *Tamarindus indica* (Chinch*), Azadirachta indica* (Neem), *Cymbopogon citratus* (Gavti Chaha), *Ficus religiosa* (Pippal Tree), *Ficus racemosa* (Umbar), *Ficus benghalensis* (Wad),*Mentha spicata L*. (Pudina), *Coriandrum sativum* (Coriender).

Secondary metabolites are the organic compounds that are not directly involved in the normal growth, development or reproduction of an organism. Unlike primary metabolites absence of secondary metabolites does not result in immediate death but rather in long term impairment of the organism. Secondary metabolites are often restricted to a narrow set of species within a Phylogenetic group. Secondary metabolites plays important role in determination of antioxidant activity (Kaur and Kapoor, 2002) [6].

A growing body of evidence suggest that dietary compounds that have significant antioxidant capacity may play a major role in explaining some of the benefits of regularly consuming fruits and vegetables. Fruits and vegetables contains many hundreds of compounds with potential antioxidant activity, including the antioxidant, vitamins C & E, carotenoids,

chlorophylls and a wide variety of antioxidants phytochemicals such as simple phenolic compounds, flavonoid glycosides and in some foods, complex polymeric tannis (e.g. Procyanidins and Gallotannins).

On the basis of their solubility, antioxidants can be roughly classified into two groups: hydrophilic antioxidants, comprising vitamin C and many of the polyphenolic compounds and lipophilic compounds, predominantly consisting of vitamin E, carotenoids and chlorophylls. Compounds includes in the classes of polyphenols and carotenoids have different degrees of solubility. The solubility of polyphenols varies according to the molecular weight and the degree of glycosylation, acylation (e.g. galloyl groups) or estrification; in particular, water solubility increases with increasing glycosylation. Regarding the solubility of lipophilic compounds, owing to their structural features, chlorophylls and more water soluble than carotenoids. In recent years the evaluation of the total antioxidant capacity of foods has receive much attention, since this index takes into account the antioxidant capacity of single compounds present in a food as well as their potential synergistic and redox interactions and may be related to efficiency of dietary protection against gastric cancer or beneficial effects against inflammatory processes (Kaur and Kapoor, 2002) [6].

Fruits and vegetables have had conferred on them the status of functional foods. They seem to be capable of delivering health benefits besides fulfilling physiological needs. Routine or habitual consumption of fruits and vegetables confers significant benefits to human health. Epidemiological data as well as *in vitro* studies strongly suggest that foods containing phytochemicals with anti-oxidation potential have strong protective effects against major disease risks including cancer and cardiovascular diseases. The protective action of fruits and vegetables has been attributed to the presence of anti-oxidants, especially anti-oxidant vitamins including ascorbic acid, a-tocopherol and b-carotene. However numerous studies have conclusively shown that the majority of the anti-oxidant activity may be from compounds such as flavonoids, isoflavone, flavones, anthocyanin, catechin and isocatechin rather than from Vitamin C, E and b-carotene (Steinberg, 1991, Kaur & Kapoor, 2001).

By considering above points in the view to determine antioxidant activity of different medicinal plant an experiment entitled "Studies on superoxide anion radical scavenging activity, antioxidant activity, and reducing power of methanolic and aqueous extract of different medicinal plants" was conducted at department of Biochemistry and molecular biology, MGM College of Agricultural Biotechnology, Gandheli, Aurangabad with following objectives 1. To formulate aqueous and methanolic extract of different medicinal plants. 2. To study antioxidant activity, superoxide anion radicial scavenging activity and reducing power of methanolic and aqueous extract of different medicinal plants.

Materials and Methods

The details of various materials and methods were adopted during the course of present investigation was narrated in this under appropriate heads:

Experimental site

The experiment was conducted in Department Biochemistry and Molecular Biology laboratory MGM College of Agricultural Biotechnology, Gandheli, Aurangabad during 2016-17 summer session.

Statistical design: Factorial Randomized Block Design (FRBD)

No. of treatments: 20 (Combination of 10 different medicinal plants & 2 extracts)

No. of replication: 03

Table. 1: Treatment Details: Factor 1:- Medicinal plants used for formulation of extracts

S. No.	Symbol	Treatment Details
1	T_1	*Phyllanthus emblica* (Amla) fruit pulp
2	T_2	*Garcinia indica* (Aamsul) fruit pulp
3	T_3	Tamarindus indica (Chinch) fruit pulp
4	T_4	*Azadirachta indica* (Neem) fruit pulp
5	T_5	*Cymbopogon citratus* (Gavti Chaha) fruit pulp
6	T_6	*Ficus religiosa* (Pippal Tree) fruit pulp
7	T_7	*Ficus racemosa* (Umbar) fruit pulp
8	T_8	*Ficus benghalensis* (Wad) fruit pulp
9	T_9	*Mentha spicata L.* (Pudina) fruit pulp
10	T_{10}	*Coriandrum sativum* (Kothimbir) fruit pulp

Table 2: Factor 2: Types of extracts

S. No.	Symbol	Treatment Details
1	E_1	Methanolic Extract
2	E_2	Aqueous Extract

Collection of fruits

The fresh fruit of *Phyllanthus emblica* (Amla), *Garcinia indica* (Aamsul),*Tamarindus indica* (Chinch*), Azadirachta indica* (Neem), *Cymbopogon citratus* (Gavti Chaha), *Ficus religiosa* (Pippal Tree), *Ficus racemosa* (Umbar), *Ficus benghalensis* (Wad),*Mentha spicata L*. (Pudina), *Coriandrum sativum* (Kothimbir) were collected from the local area of Aurangabad Maharashtra.

Preparation of methanolic extracts

Medicinal plants (Amla, amsul, chinch, neem, gavti chahaa, papal, umber, wad, pudina, kothimbhir) were collected. The fruits were washed in tap water and air dried at room temperature. Dried fruits were washed in tap water and air dried at room temperature. Dried fruits 5 gram by quantity was grind to produce fine homogenous mixture. The fine fruit mixture was soaked in 40 ml of 95% Methanol at room temperature for 72 hours in dark. The solution was then filtered through Whatman filter paper and evaporated to dryness using rotary evaporator at temperature below 40^0 C and final filtrate was used for further procedure. (Kanatt *et al*., 2007) [4].

Preparation of aqueous extract

The aqueous extract of the medicinal plants were obtain by adding 10 ml of boiling water to 5 gm of powdered plant material in glass flask and incubated at room temperature for 8 hours on a rotating shaker (200 rpm). The aqueous extract was filtered using Whatman no. 1. (Ao *et al*., 2008) [1].

Antioxidant activity (DPPH Free radical scavenging activity) of extract

DPPH assay was performed according to the method of Yamaguchi, Takamura, Matoba, and Terao (1998).The diluted extract (200 µl) was mixed with 800 µl of Tris–HCl buffer (100 mm, pH 7.4). To this was added 1 ml of 500 µM DPPH in ethanol (final concentration of 250 µM) and the whole vortexed vigorously. The tubes were then incubated at room temperature for 20 min under dark conditions and the absorbance was measured at 517 nm (Yamaguchi *et al*.,

1998). Percent DPPH-scavenging activity was calculated as: [(Control absorbance - Extract absorbance) / (Control absorbance)] × 100.

Superoxide anion radical-scavenging activity of extract

Superoxide anion-scavenging activity determined according to the method of Liu, Ooi, and Chang (1997) with some modifications. The reaction mixture consisted of 1 ml of NBT (156 µM in 0.1 M potassium phosphate buffer pH 7.4), 1.0 ml of NADH (468 µM in 0.1 M potassium phosphate buffer pH 7.4) and 0.5 ml of an appropriately diluted sample. The reaction was initiated by addition of 100 µl of PMS (60 µM in 0.1 M potassium phosphate buffer pH 7.4) to the mixture.

The tubes were incubated at ambient temperature for 5 min and the absorbance was measured at 560 nm. Decreased absorbance of the reaction mixture indicated increased superoxide anion-scavenging activity (Liu *et al*., 1997).

The percentage inhibition of superoxide anion generation was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_S) / A_0] \times 100,$$

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

Reducing power of extract

The reducing power of the extracts was determined according to the method of Oyaizu (1986). An aliquot (2.5 ml) was mixed with 2.5 ml of 200 mm sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide and the mixture was incubated at 50^0C for 20 min. Ten percent TCA (2.5 ml) was added and the mixture was centrifuged at 650g for 10 min. The upper layer (5 ml) was mixed with 5 ml of distilled water and 1 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm (Oyaizu, 1986).

Result and Discussion

The results obtained in the presents investigation on Studies on superoxide anion radical scavenging activity, antioxidant activity, and reducing power of methanolic and aqueous extract of different medicinal plants are presented under following heads.

1.1 Antioxidant activity

Data on mean antioxidant activity recorded after DPPH radical scavenging assay presented in Table 3.

Table 3: Percentage of antioxidant activity shown by different medicinal plants

Extract (Factor B) / Medicinal plants (Factor A)	Methanolic Extract E_1	Aqueous Extract E_2
T_1	74.34	53.33
T_2	68.32	49.41
T_3	72.55	68.32
T_4	55.96	49.02
T_5	59.79	44.04
T_6	57.84	43.47
T_7	61.12	40.39
T_8	55.34	45.19
T_9	60.01	50.77
T_{10}	57.63	45.76

	SE ±	CD at 1%
Factor A	0.055	0.474
Factor B	0.055	0.212
A x B	0.175	0.671

1) Effect of different medicinal plants

Data presented in Table 3 indicated that the mean antioxidant activity was influenced significantly by different medicinal plants. Methanolic Extract of *Phyllanthus emblica* (Amla) (T_1) showed highest antioxidant activity (74.34%) among all other medicinal plants and treatment (T_1E_1) found significantly superior over rest of the treatments Viz.T_2 to T_{10}.The antioxidant activity of treatments T_1,T_2,T_3,T_4,T_7,T_8 found significantly superior. The antioxidant activity of treatments T_5, T_6, T_9, T_{10} found non-significant or which are at par.

2) Effect of different extracts

The antioxidant activity was significantly influenced due to different extracts. Data presented in Table 3.1 indicated that the Methanolic Extract of medicinal plants shows highest antioxidant activity as compare to the aqueous extract of medicinal plants. The interaction of methanol with medicinal plants is responsible for the antioxidant activity. The Methanolic Extract of *Phyllanthus emblica* (Amla) (T_1E_1) showed highest antioxidant activity (74.34%) among all other medicinal plants and found significantly superior over the rest treatment Viz.T_2 to T_{10}.

3) Interaction effects

It was found that the antioxidant activity of methanolic extract enhanceses as compare to aqueous extract. Interaction (A×B) effect on antioxidant activity were found significant. Treatment combination (T_1E_1) recorded significantly higher antioxidant activity (74.34%) compared to all other treatments tried in the experiment. Similar findings also reported by Ao C, Li A, Elazaawely AA, Xuan DT, Tawata S (2008) [1] during evaluation of antioxidant and antibacterial activities of *Ficus Microcarpa*, Liu X, Cui C, Zhao M, Wang J, Luo W, Yang B, Jiang Y. (2008) [7]. While Identification of phenolics in the fruit of *Phyllanthus emblica L.* and their antioxidant activities, Veerapur VP *et al*. (2007) [9] in *ficus racemosa*.

3.2 Superoxide anion radical-scavenging activity of extract

Data on mean Superoxide anion radical-scavenging assay depicted in Figure 1.

1) Effect of different medicinal plants

Data depicted in Figure 1 indicated that the Methanolic Extract of *Cymbopogon citratus (Gavti Chaha)* (T_5) showed highest Superoxide anion radical-scavenging activity (65.92%) among all other medicinal plants and treatment (T_5E_1) found significantly superior over the rest other treatments.The Superoxide anion radical-scavenging activity of treatments T_1 to T_{10} found significantly superior.

The *Cymbopogon citratus (Gavti Chaha)* (T_5) Superoxide anion radical-scavenging activity has highest compare to rest other treatments.

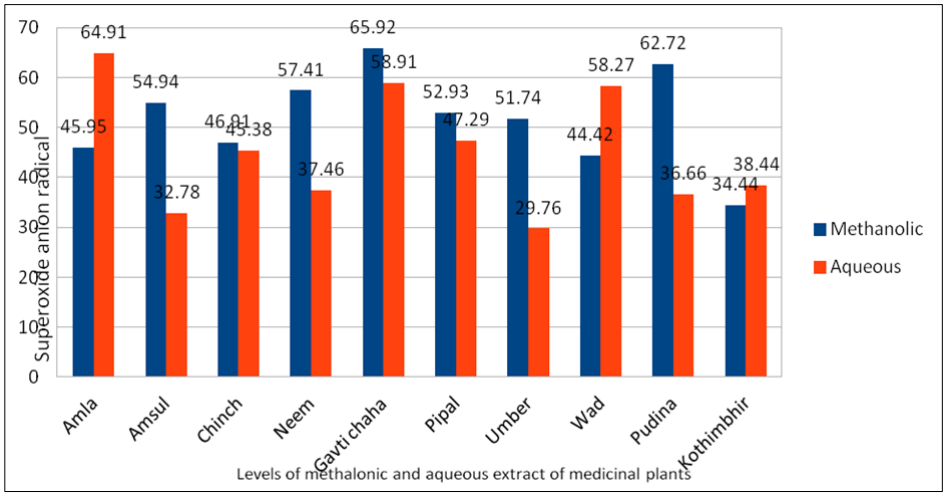

Fig 1: Percentage of Superoxide anion radical-scavenging activity shown by different medicinal plants.

2) Effect of different extracts

The Superoxide anion radical-scavenging activity was significantly influenced due to different extract. Data presented in figure 2 indicated that the Methanolic extract of medicinal plants shows highest superoxide anion radical-scavenging activity as compare to the aqueous extract of medicinal plants. The Methanolic Extract of *Cymbopogon citratus (Gavti Chaha)* (T_5E_1) showed highest Superoxide anion radical-scavenging activity (65.92%) among all other medicinal plants and found significantly superior over the rest other treatments.

3) Interaction effects

The interaction of methanol with medicinal plants is responsible for the superoxide anion radical-scavenging activity. Interaction (A×B) effect on Superoxide anion radical-scavenging activity were found significant. Treatment combination T_5E_1 *Cymbopogon citratus (Gavti Chaha)* recorded significantly higher activity (65.92%) compared to all other treatments tried in the experiment. It was found that the superoxide anion radical-scavenging antioxidant activity of methanolic extract enhances as compare to aqueous extract.

3.3Reducing power of extract

Data on mean Reducing power of extract is depicted in Figure 2

1) Effect of different medicinal plants

Data presented in figure 2 indicated that the Methanolic Extract of *Phyllanthus emblica* (Amla) (T_1) showed highest reducing power of extract (3.43) among all other medicinal plants and found significantly superior over the rest treatment Viz.T_2 to T_{10}. The reducing power of extract of treatments T_1, T_2, T_3, T_4, T_5, T_7, T_9, T_{10} found significantly superior. The reducing power of extract of treatments T_6 andT_8, found non-significant or which are at same level at par.

The *Phyllanthus emblica* (Amla) has highest reducing power of extract compare to rest other treatments.

2) Effect of different extracts

The reducing power of extract was significantly influenced due to different extract. Data presented in figure 2 indicated that the Methanolic Extract of medicinal plants shows highest reducing power of extract as compare to the aqueous extract of medicinal plants. The methanolic extract of *Phyllanthus emblica* (Amla) (T_1E_1) showed highest reducing power of extract (3.43) among all other medicinal plants and found significantly superior over the rest treatment Viz.T_2 to T_{10}.

3) Interaction effects

Interaction (A×B) effect on reducing power of extract were found significant. Treatment combination T_1E_1 recorded significantly higher reducing power of extract (3.43) compared to all other treatments tried in the experiment. It was found that the reducing power of extract of methanolic extract enhances as compare to aqueous extract. The interaction of methanol with medicinal plants is responsible for the reducing power of extract**.**

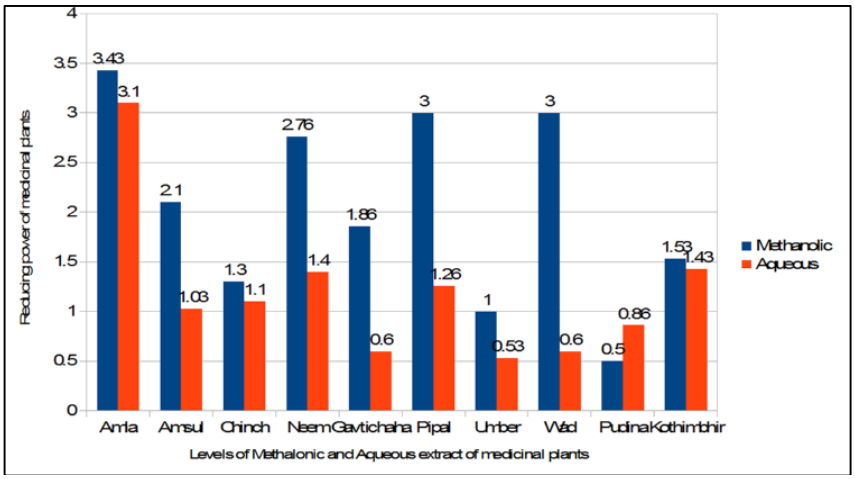

Fig 2: Reducing power shown by different medicinal plants.

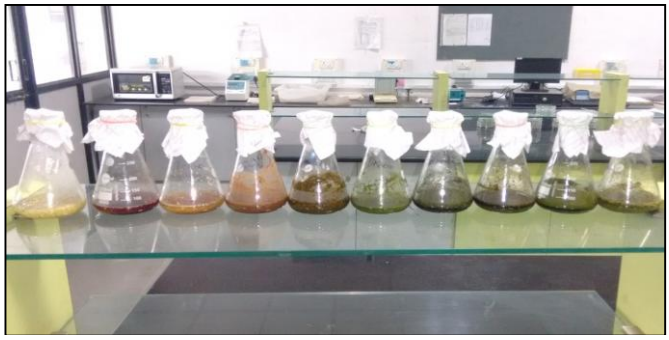

Plate 1: Methanolic extract of different medicinal plant sample

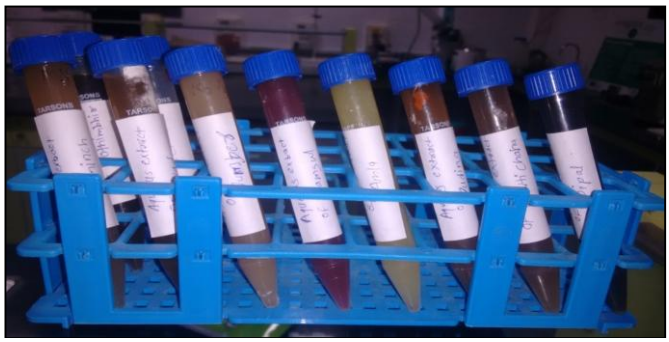

Plate 1: Aqueous extract of different medicinal plants

Outcome of the Research

After completion of project the final outcome of investigation is as following:

The Methanolic extract of *Phyllanthus emblica* (Amla) has highest antioxidant activity 74.34%. The methanolic extract has significantly superior antioxidant activity compare to aqueous extract of medicinal plants. The Methanolic extract of *Cymbopogon citratus* (Gavti Chaha) has highest superoxide anion radical scavenging activity 65.92%. The methanolic extract has significantly superior superoxide anion radical scavenging activity compare to aqueous extract of medicinal plants. The Methanolic extract of *Phyllanthus emblica* (Amla) has highest reducing power activity 3.43. The methanolic extract has significantly superior reducing power activity compare to aqueous extract of medicinal plants. Emblica has highest antioxidant potential.

Conclusion

The antioxidant activity of the medicinal plants extract and the standard were assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) free radical activity by modified method. The dilute working solutions of the test extract were prepared in methanol. Ascorbic acid was used as standard in 1-100µg/ml solution. 0.002% of DPPH was prepared in methanol. These solution mixtures kept in dark for 30 min and optical density was measured at 517nm using Cecil-Elect Spectrophotometer. Methanol (1ml) with DPPH solution (0.002%, 1ml) was used as a blank. The optical density was recorded and percentage inhibition of was calculated using the following formula:

$$\% \text{ Inhibition} = [(A_0 - A_S) / A_0] \times 100,$$

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

Further studies are required to better evaluate the effect of these extracts. *In vivo* clinical testing is essential to confirm *in vitro* results. As the evidence base of the medicinal plants evolves, so will our understanding. More research into medicinal plants extracts and antioxidant synergistic effect. The various serious diseases such as neurodegenerative disorders, cancer, liver cirrhosis, cardiovascular diseases, atherosclerosis, cataracts diabetes and inflammation can be controlled using medicinal plants.

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