IJISET - International Journal of Innovative Science, Engineering & Technology, Vol. 2 Issue 6, June 2015.
www.ijiset.com
ISSN 2348 – 7968

Invitro study of antioxidant activities of medicinal plant extracts using $KMnO_4$ and DPPH-a comparison

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Abstract

Medicinal plants from 11 different families have been chosen in our study for their antioxidant activity . Antioxidants reduce the oxidative stress by terminating the free radical chain reactions .Antioxidant activity is expressed in terms of EC50 values .In our present paper, the antioxidant properties & the free radical scavenging action of the plant extracts , have been analysed using an oxidizing agent such as potassium permanganate (by colorimetry) and also using free radical 2,2-Diphenyl-1-picryl hydrazyl (DPPH) in its radical form by (Spectrophotometer). $KMnO_4$ activity has an absorption band at 520nm and DPPH at 517 nm. An attempt was made to compare the antioxidant activities evaluated by both the methods in our study and were found to be comparable

Keywords **:** Antioxidant activity ,EC50 ,$KMnO_4$ & DPPH

1. Introduction

Oxidative stress is the main cause for the chronic degenerative diseases. During oxidation, free radicals are formed which damage the cells . Antioxidative defence mechanism is necessary to diminish the action of free radicals (1,2).A great number of medicinal plants contain chemical compounds which exhibit antioxidant properties (Young & woodside , 2001) . In our present work an attempt was made to assess and compare the antioxidant activity in various medicinal plants using $KMnO_4$ (Redox reaction method) and DPPH (free radical Scavenging method) . $KMnO_4$ is a powerful oxidizing agent in sulphuric acid medium which gives purple color at λmax 520nm which becomes colorless in presence of antioxidants.

DPPH (2,2-Diphenyl-1-picryl hydrazyl) is a stable free radical due to the delocalization of the free electron and gives a violet color at λmax 517nm (spectrophotometer) which becomes colorless in the presence of antioxidants(3). Using $KMnO_4$ (Redox reaction method) and DPPH ,free radical scavenging activity was evaluated through the determination of EC50 values EC50 refers to half maximal effective concentration & in turn refers to the concentration of a drug antibody or toxicant which induces a response halfway between the base line and maximum after a specified exposure time .It is commonly used as a measure of drug potency . The EC50 of graded dose response curve represents the concentration of compound where 50 % of its maximal value is observed (4) .

2 Methodology :

2.1 Medicinal Plant Materials :

The medicinal plants were collected from botanical garden at Muffakhamjah College of Engineering and Technology, Banjarahills , Hyderabad , Telangana , India . The plant materials were cleaned and the details are shown In Table - I .

2.2 Method of Extraction for $KMnO_4$ (redox reaction method) :

Fresh plant materials were cleaned and ground to fine paste ,squeezed using muslin cloth and filtered using whattman filter paper no:42 to remove any suspended particles, 1 ml of pure extract was diluted upto 100ml using 100ml distilled water .

2.3 Method of Extraction for DPPH (free radical Scavenging method) :

Plant materials were air dried at room temperature & ground to a uniform powder, Ethanol Extracts were prepared by soaking known amount of plant materials in 1lt. of ethanol at room temperatue. The extraction procedure was completed by filtering through whattman Filter paper no : 42 and then concentrated using a rotary evaporator at 40^oc . 1 ml of the extract was diluted upto 100 ml using distilled water.

2.4 Antioxidant activity (Potassium Permangante free radical scavenging activity) Determination :

The antioxidant activity of plant extracts was examined on the basis of the scavenging effect on Standard Potassium permanganate free radical activity , (5) (Brace et,al ,2002). Potassium Permanganate was standardized using Ferrous ammonium sulphate . The method is based on redox reaction between medicinal plant extracts & Potassium permanganate in Sulphuric acid medium leading to sample discoloration . Potassium permanganate is a strong oxidizing agent and permanganate ion (Mno_4^-) is dark purple in color . Reduction of purple permanaganate ion to colorless Mn^{2+} ions occurs in acidic medium, in the presence of medicinal plants extracts and no additional indicators are needed. Standard Potassium permanganate solution 0.00048M was added to each of the plant extracts (shown in Table-1) in the pressure of Sulphuric acid medium and optical density values were noted using colorimeter at 520nm, wavelength.Selection of wavelength was done and Beer-Lambarts law obeyed (6). Distilled water was used to set the absorbance to zero and the instrument was calibrated .Optical density values of Potassium permanganate with plant extracts were used in evaluating EC50 values .

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2.5 Antioxidant activity (DPPH free radical scavenging activity) Determination :

The free radical scavenging activity against 1,1 Diphenyl 2-picryl hydrazyl (DPPH) was evaluated and the antioxidant activity was determined . DPPH an antioxidant assay produces a violet solution in ethanol (7). The DPPH free radical is stable at room temperature & is reduced to colorless ethanol solution in the presence of an antioxidants and analyzed spectrophotometrically. Ethanolic solution of DPPH (0.0005M) was added to the plant extracts and absorbance measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance to zero and the instrument was calibrated.

3. Tables, Figures and Equation

English name	*Botanical name*	*Family name*	*Part used*	*Therapeutic uses*
Neem	Azardirachta Indica	Meliaceae	Leaves	Intestinal normal ,liver problems, skin diseases etc
Prickly chaff	Achyranthus aspera	Amaranthceae	Leaves	Obstetrics & gynecology, to treat malaria
Holy Basil	Ocimum sanctum	Lamiacea	Leaves	Antibacterial ,skin, allergies gastric troubles etc
Bengal Quince (Golden Apple)	Aegle Mermelos	Rutaceae	Leaves	Gynecological Disorders , urinary ailments , Gastrointestinal ailments etc
aloevera	Aloe Indica Royle	Xanthorrhoeaceae	Leaves	Digestion Gynecological Disorders ,Skin Diseases.etc
Eucalyptus Eucalyptus	Eucalyptus obligua	Myrtacaea	Leaves	Respiratory disorders,as an insecticide etc
Curry leaves	Murraya koenigii	Rutaceace	Leaves	Prevents Anemia, Hair Treatments ,Digestion etc
Tridax daisy (or) Coat Buttons	Tridax Procumbers	Asteraceae	Leaves	Insecticidal,anti inflammatory activity,antiviral,antibiotic.etc
False Daisy	Wedelia Calendulaceaae	Asteraceae	Leaves	Hair & Skin Treatments
Mint	Mentha Longigolia	Lamiaceace	Leaves	Irritable bowel Syndrome, stomach ache ,nausea etc
Paan	Piper betle	Piperaceae	Leaves	Mouth freshner,diuretic properties, nervous pains, stomach disorders,etc
Coriander	Corriandrum Sativum	Apiaceae	leaves	leveling blood glucose level in diabetics, nausea ,vomiting,etc
Guava	Psidium guajava	Myrtaceae	Leaves	Anti ageing diets, Prevent anemia, act against cholesterol, intestinal cramps ,etc
Karela	Momoridica Charantia	Cucurbitacae	Leaves	Antidaibetic,laxative,emetic,anthalmintic agent, lowering blood glucose level

Table -1 Characteristics of the medicinal plants used

Table : 2 EC50 values as evaluated by $KMnO_4$ and DPPH methods

S.no	*English name of Medicinal Plants*	*Botanical name*	*EC50 values (µg/ml) by redox method*	*EC50 values (µg/ml) DPPH method*
1.	Neem	Azardivarchta Indica	0.0024	0.0018
2.	Prickly chaff	Achyranthus aspera	0.0034	0.0042
3.	Holy Basil	Ocimum sanctum	0.0024	0.0018
4.	Bengal Quince	Aegle Mermelos	0.0072	0.0076
5.	aloevera	Aloe Indica Royle	0.0039	0.0051
6.	Eucalyptus	Eucalyptus obligua	0.0039	0.0051
7.	Curry leaves	Murraya koenigii	0.0024	0.0018
8.	Tridax daisy (or) Coat Buttons	Tridax Procumbers	0.0039	0.0051
9.	False Daisy	Wedelia Calendulaceaae	0.0115	0.0018
10	Mint	Mentha Longigolia	0.0068	0.0074
11.	Paan	Piper betle	0.0052	0.0056
12.	Corriander	Corriandrum Sativum	0.0039	0.0051
13.	Guava	Psidium guajava	0.0124	0.0191
14.	Karela	Momoridica Charantia	0.0034	0.0042

Figure -1: Plots of O.D vs. EC50 values (using $KMnO_4$)

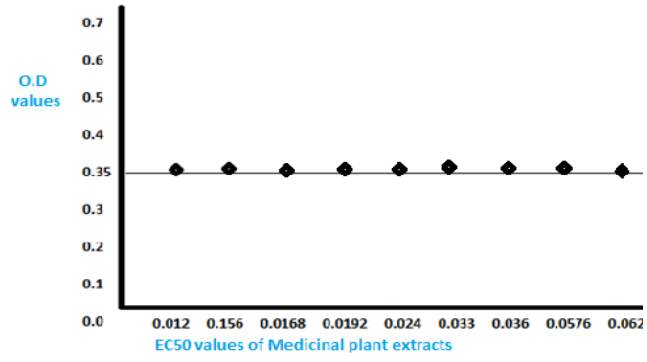


Figure -2: Plots of O.D vs. EC50 values (using DPPH)

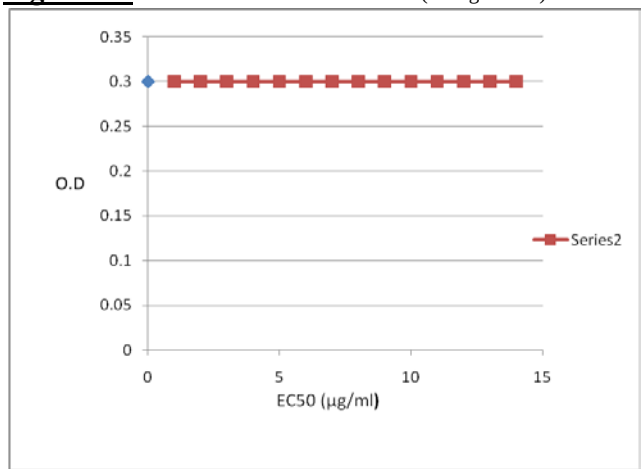
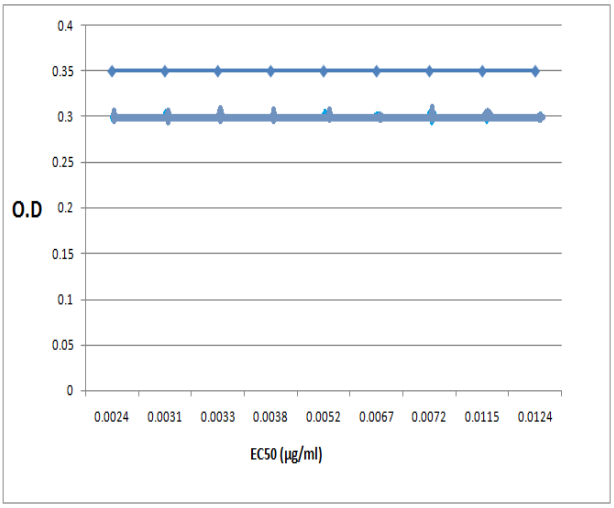


Figure-3: Comparitive graph of DPPH and Kmno4



Results and Discussion :

In the present study the antioxidant activities of the medicinal plants (shown in Table -1) are evaluated colorimetrically (using potassium permanganate) and spectrophotometrically (using DPPH).

Potassium permanganate is a powerful oxidizing agent and anti oxidant activities of the plant extracts were determined by redox reaction method (kolear.et.al). Standard potassium permanganate solution (0.00048M)

ISSN 2348 – 7968

was found to give maximum absorbance at 520nm. Hence all observations were done at 520nm . The total antioxidant activity studies were done by Redox reactions colorimetrically and EC50 values for each plant extract were obtained from the optical density of potassium permanganate (0.66) , concentration of potassium permanganate (0.00048 M)(Table-2,Figure-1).
The total anti oxidant activity studies were done by free radical scavenging action by DPPH (Spectrophotometrically) and EC50 values for each plant extract were obtained from the O.D of DPPH (0.59) concentration of DPPH (0.0005M) (Table-2,Figure-2).

4. Conclusion :

It was observed that the EC50 values of the plant extracts done by both methods were found to be comparable & reproducible(Figure-3).It was noted that the stock solutions of the orginal free radical (DPPH) do slowly deteriorate(8) .Therefore Potassium permanganate method was recommended.Potassium permanganate method was found to be much simpler ,less expensive & less time consuming with proper filter chosen .

Acknowledgments

The authors are thankful to the Head, Department of Chemistry, Principal, Director of Muffakham Jah College of Engineering and Technology for providing all the facilities to carry on the research work.We would also like to thank Mr.Md Rashed Ali for his help during our work.

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