Swarna Latha G. *et al*. / *International Journal of Phytopharmacology*. *5*(4), 2014, 288-292.

e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF FLOWER OF *Commelina clavata*

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ABSTRACT

Antioxidant activity of methanolic extract of whole plant of *Commelina clavata* was evaluated using the free radical scavenging activity of the Hydrogen peroxide radical scavenging activity, Reducing power assay and Phosphomolybdenum method. The phytochemical studies on the methanolic extracts of *Commelina clavata* have revealed the presence of phenolic compounds like Flavonoids and and terpinoids. In the method H_2O_2 scavenging activity of hydrogen peroxide method the absorbance values of *Commelina clavata* extracts show lower activity with that of standard values. Here ascorbic acid is used as standard. In this reducing power method the absorbance values of *Commelina clavata* show lower activity with that of standard values. Here ascorbic acid is used as standard. In this phosphomolybdenum method increase in the reaction mixture indicates increasing reducing power. For *Commelina clavata* extracts the absorbance values equal at 40 µg and from 20µg, 60 µg, 80 µg and 100 µg it shows this is having decreasing reaction. So *Commelina clavata* extract show comparable activity at 40 µg. Here DMSO is used as standard drug. The methanol extracts of whole plant of *Commelina clavata* was exhibited less potent anti-oxidant activity in H_2O_2 scavenging hydrogen peroxide method and reducing power method and potent anti-oxidant activity in Phosphomolybdenum method.

Key words: Reducing power assay, Phosphomolybdenum method, *Commelina clavata*.

INTRODUCTION

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine of Allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as on important source of future medicine and therapeutics (Mcchesney JD *et al*., 2007).

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The World Health Organization (WHO, 1991) has recently defined traditional medicine (including herbal drugs) as comprising therapeutic practices that have been existence, often for hundreds of years, before the development and spread of modern medicine and are still in use today. Or say, traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous system of medicine. Indeed, more than 90% of current therapeutic classes derive from a natural product prototype and interestingly, even today, roughly two-thirds to three-quarters of the world's population relies upon medicinal plants for its primary pharmaceutical care. Those "medicinal plants" are either preparations of or natural product substances from plants that have potential utility as pharmaceutical agents (Balanus MJ, Kinghorn AD, 2005).

MATERIALS AND METHODS

Chemicals

Ammonium molybdate, potassium ferricyanide , Phosphate Buffered Saline (PBS)sulphuric acid,ferric chloride,potassium ferricyanide . Ethyl alcohol and Dimethyl Suphoxide (DMSO) were procured from SD Fine chemicals,

Plant materials

The plant materials such as whole plant of *Commelina clavata* was collected from arid lands of Anantapur district of Andhra Pradesh state. The plants materials were then identified and authentified by Dr. Venkatapathi Raju, Professor, Department of Botany, Sri Krishnadevaraya University, Anantapur. A voucher specimen of *Commelina clavata*, has been deposited in the Herbarium of the Department of botany for the further reference

Preparation of Extracts

The freshly collected plant materials were washed, shadow dried and then dried in hot air oven at a temperature not more than 50°C. The dried materials were coarsely powdered using an electric blender. Powdered materials (500g) were then packed in soxhlet apparatus and successively extracted with Ethanol and methanol. Each time before extraction with the next solvent, the powdered materials were dried in hot air oven at below 50°C. Finally extracts were concentrated in rotary evaporator at a temperature not more than 50°C and then, dried under vacuum dessicator. The dried extracts thus obtained were used for further experiments. In the current research we have used the Ethanolic and methanolic extracts of *Commelina clavata*.

Phytochemical studies

Preliminary phytochemical screening was done using the specified protocols for the qualitative analysis of Alkaloids, carbohydrates, fixed oils, flavonoids, glycosides, phyto sterol /terpenoids, saponins, and tannins/phenols (Wyllie A, Duvall E, 1992; Vinod R, 2002; Harborne JB, 1973).

Antioxidant activities

Phytochemical investigation will be a useful tool for the identification and authentication of the plant for industrial and further research purpose. Total phenol content of a tested material is related to the antioxidant activity. Antioxidants, which can scavenge free radicals, have an important role in pharmacological systems. Antioxidants are emerging as prophylactic and therapeutic agents. Hence, antioxidant was also evaluated for the potent extract.

There are a number of clinical studies suggesting that the antioxidants in fruits, vegetables, tea and red wine are the main factors for the observed efficacy of these foods in reducing the incidence of chronic diseases including heart disease and some cancers. The free radical scavenging activity of antioxidants in foods has been substantially investigated and reported in the literature by Miller *et al*., (2000).

In-vitro models for evaluating antioxidant activities

Herbal plants are known to contain a variety of antioxidants. Numerous substances have been suggested to serve as antioxidants. It has been revealed that various phenolic antioxidants, such as flavonoids, tannins, coumarins, xanthones and more recently procyanidins scavenge radicals dose-dependently, thus they are viewed as promising therapeutic drugs for free radical pathologies (Mc Comb RB, Bowers GN, 1972). Reactive oxygen species (ROS) and free radicals such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are constantly formed in the human body by normal metabolic action and have been implicated in the pathologies of certain human diseases, including cancer, ageing, diabetes and atherosclerosis. Their action is opposed by a balanced system of antioxidant defenses including antioxidant compounds and enzymes. Upsetting this balance causes oxidative stress, which can lead to cell injury and death. Current research into free radicals has confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases, cancers and neurodegenerative diseases. Therefore, much attention has been focused on the use of natural antioxidants to inhibit lipid peroxidation, or to protect the damage of free radicals (Harborne JB, 1973).

Scavenging of hydrogen peroxide

A solution of H_2O_2 (20mm) was prepared in phosphate buffer saline (PBS, PH 7.4). Various concentration (10µg-100µg) of standard and extracts was prepared, 1ml of the extract and standard was dissolved in methanol in a separate volumetric flask and to this solution 2ml of H_2O_2 solution in PBS was added, the absorbance was measured at 230nm, after 10min against blank solution.

Determination of Reducing Power

Method based on the principle of increase in the absorbance of the reaction mixture. Increase in the absorbance indicates increase in anti-oxidant activity. Different concentration of extracts (20µg-100µg) in 1ml of distilled water were mixed with 2.5ml of phosphate buffer (0.2m; pH 6.6) & 2.5ml of potassium ferricyanide [$K_3Fe(CN)_6$] (1%), the resulting mixture was incubated at 50^{o}C for half an hour. Then, 2.5ml of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged at 3000rpm for 10min. Finally 2.5ml of upper layer solution was mixed with 2.5ml of distilled water and 0.5ml of $FeCl_3$ (0.1%) were added. The absorbance was

measured at 700nm in UV-Vis spectrophotometer against blank. Increasing of the reaction mixture indicates increasing reducing power.

Estimation of Phospho molybdenum

In this method quantitative determination of anti-oxidant capacity, through the formation of phosphor molybdenum complex. The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate Mo (V) complex at acidic pH. An aliquot of 0.3ml of sample solution containing a reducing species in DMSO was combined in a test tube with 3ml of reagent solution (0.6m H_2SO_4, 28mm sodium phosphate and 4mm ammonium molybdate) then the tubes were covered with aluminium foil and kept in a water bath at 95^{O}c for 90min. Then the samples were cooled to room temperature, absorbance of each solution was measured at 695nm against blank. The total anti-oxidant was expressed as mm equivalent to ascorbic acid. The results are tabulated in -respectively.

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Ash values

The ash values such as total ash, acid insoluble, water soluble and sulphated ash of whole plant of *commelina clavata*a was determined and results was shown in Table: 1.

Elemental analysis

The implications of heavy metals are becoming very vital in now- a days. The ingestion of these might be beneficial or harmful depending up on the nature of the element present. The toxic elements might develop cumulative or genetic toxicity and would also be carcinogenic in nature.

Toxic and heavy metal content

The toxic elements like arsenic, lead, palladium, mercury and cadmium were established by inductively coupled plasma optical emission spectrometer (ICP-OES) and results were tabulated in Table 2.

Extractions

The percentage yields of the extractives of whole plant of C*ommelina clavata* was observed and results are shown in Table 3.

***In-vitro* models for evaluating antioxidant activities**

The antioxidant activity of methanolic extract of whole plant of *Commelina clavata* was carried out using following methods and the results are recorded in the following Tables: 5,6 and 7.

1. Hydrogen peroxide Assay scavenging profile
2. Reducing power method
3. Phospho molybdenum method.

Table 1. Ash value of *Commelina clavata*

Types of Ash	%yield of *Commelina clavata*
Total ash	5.35
Acid insoluble ash	1.30
Water soluble ash	2.04
Sulphated ash	6.05

Table 2. Toxic and Heavy Metals content

Metals	*Commelina clavata*
Arsenic	0.05
Lead	0.02
Mercury	0.00
Cadmium	0.06

Each value represented in ppm

Table 3. Extractive values in different solvents

Extractives	%yield of *Commelina clavata*
Ethanol	11.0
Methanol	12.3

Table 4. Preliminary phytochemical Screening of *Commelina Clavata* extract

Type of phyto chemical constituents	*Methanol extract of Commelina clavata*
Alkaloid	+ve
Carbohydrates	+ve
Glycosides (Anthraquinone, cardiac)	-ve
Saponin glycosides	-ve
Proteins	+ve
Volatile oils	-ve
Fats and fixed oils	-ve
Flavonoids	+ve
Terpenoids	+ve

+ve: Indicates the presence of phytochemical constituents.
-ve: Indicates the Absence of phytochemical constituents.

Table 5. Hydrogen peroxide assay Scavenging profile

Concentration/Absorbance	10µg	25 µg	50 µg	75 µg	100 µg
Ascorbic acid	1.517± 0.06	1.554±0.04	1.614±0.06	1.623±0.07	1.659± 0.08
CCM	0.890 ± 0.03	1.060 ± 0.07	1.240±0.05	1.460±0.05	1.810± 0.03

Table 6. Determination of Reducing Power

Concentration/ Absorbance	20 µg	40 µg	60 µg	80 µg	100 µg
ASCORBIC ACID	1.770± 0.06	2.132± 0.05	4.943± 0.02	4.945± 0.02	4.947±0.02
CCM	0.890 ± 0.05	1.040 ± 0.08	1.340 ± 0.07	1.570 ± 0.05	1.830 ± 0.06

Table 7. Estimation of Phosphomolybdenum

Concentration/ Absorbance	**20** µg	**40** µg	**60** µg	**80** µg	**100** µg
DMSO	0.126± 0.04	0.027± 0.01	0.297± 0.03	0.385± 0.02	0.520± 0.01
CCM	0.113 ± 0.05	0.133 ± 0.04	0.106 ± 0.05	0.142 ± 0.03	0.112 ± 0.04

DISCUSSION AND CONCLUSION

Commelina clavata, ash content. It is due to the presence of high inorganic content. However, the ash content is possibly due to the Na^+ and Ca^{2+} salts which are not harmfull (Sahito SR, 2001). Heavy metals are being spoken out very widely in the global scenario due to the recent episodes of a few Indian Ayurvedic formulations which have been found to have heavy metals more than that of the permissible level as advised by W.H.O. and F.A.O. of U.S.A (WHO, 1980). Toxic heavy metals like arsenic, lead, cadmium and mercury were within the limit and ensure the safety of the study. Plant extract was then subjected to preliminary phytochemical screenings, and the results of these studies have shown that,CCM extract are Carbohydrates, Proteins, Flavonoids and Terpenoidal Compounds

In the method H_2O_2 scavenging activity of hydrogen peroxide method the absorbance values of CCM extracts show lower activity with that of standard values. Here ascorbic acid is used as standard. In this reducing power method the absorbance values of CCM extracts show lower activity with that of standard values. Here ascorbic acid is used as standard. In this phosphomolybdenum method increase in the reaction mixture indicates increasing reducing power. For CCM extracts the absorbance values equal at 40 µg and from 20µg, 60 µg, 80 µg and 100 µg it shows this is having decreasing reaction. So CCM extract show comparable activity at 40 µg. Here DMSO is used as standard drug.

Among all compared to three methods Hydrogen Peroxide method, Reducing Powers Method and Phosphomolybdenum Method *Commelina clavata* Plant shows activity.

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