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Reactive oxygen and nitrogen species scavenging activity of *Commelina maculata* extract-an *In vitro* study

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Abstract
Antioxidant activity of ethanolic extract of *Commelina maculata* whole plant were carried out for proving its utility in free radical mediated diseases including diabetic, cardiovascular, cancer etc. The ethanolic extract was screened for *in vitro* antioxidant activity by DPPH scavenging, total antioxidant, nitric oxide scavenging, hydroxyl radical scavenging and reducing power assay at different concentrations. Throughout the studies extract showed marked antioxidant activity. The antioxidant activity of the extract may be due to the phytochemicals present in it. The antioxidant activity was found to be concentration dependent and may be attributed to the presence of bioflavonoids content in the of *Commelina maculata.* Overall, the *Commelina maculata* extract is a source of natural antioxidants which might be helpful in preventing the progress of various oxidative stress mediated diseases including aging.

Keywords: antioxidant activity, *Commelina maculata*, radical scavenging, reactive oxygen species

Introduction

The adverse effects of oxidative stress on human health have become a serious issue. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage (Bhatia *et al.*, 2003; Peuchant *et al.*, 2004) [1, 2] and health problems (Steer *et al.*, 2002) [3]. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases (Velavan, 2011; Alma *et al*, 2003) [4, 5]. Natural and synthetic antioxidants are beneficial to free radical mediated diseases. Synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis (Grice, 1988) [6] for this reason, interest in the use of natural antioxidants has increased.

The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components (Winston, 1999) [7]. Plant and its products are rich sources of a phytochemicals and have been found to possess a variety of biological activities including antioxidant potential (Velavan *et al*, 2007 and 2015) [8, 9]. The majority of the active antioxidant constituents are flavonoids, isoflavones, flavones, anthrocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential (Prior, 2003) [10]. With this background and abundant source of unique active components harbored in plants. The chosen medicinal plant namely as *Commelina maculata* blonging to Commelinaceae family. Hence, the free radical scavenging activity of *Commelina maculata* was not evaluated. Therefore, the present study were to investigate the free radical scavenging activity of *Commelina maculata* through the free radical scavenging such as DPPH scavenging, total antioxidant, nitric oxide scavenging, hydroxyl radical scavenging and reducing power assay.

Materials and Methods

Chemicals

Nitro blue tetrazolium (NBT), ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), trichloro acetic acid (TCA), thio barbituric acid (TBA), potassium hexa cyano ferrate [$K_3Fe(CN)_6$], and L-ascorbic acid were purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Plant Materials

The leaves of *Commelina maculata* was collected in January 2019 from Tamil University, Thanjavur District, Tamil Nadu, India from a single herb. The leaves was identified and authenticated by Dr. S. John Britto, The Director, the Rabiant Herbarium and center for molecular systematics, St. Joseph's college Trichy-Tamil Nadu. India. A Voucher

specimen has been deposited at the Rabinat Herbarium, St. Josephs College, Thiruchirappalli, Tamil nadu, India.

Preparation of alcoholic extract

The leaves of *Commelina maculata* was first washed well and dust was removed from the plant. Whole plant was washed several times with distilled water to remove the traces of impurities from the plant. The whole plant was dried at room temperature and coarsely powdered. The powder was extracted with ethanol for 24 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in refrigerator until used.

Antioxidant Assay

The scavenging ability of the natural antioxidants of the plant extract towards the stable free radical DPPH was measured by the method of Shimada *et al.*, (1992) [11]. The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al.*, (1999) [12]. The scavenging activity for hydroxyl radicals was measured with Fenton reaction by the method of Yu *et al*. (2004) [13]. Nitric oxide radical scavenging activity was determined according to the method reported by Garrat (1964) [14]. The Fe3+ reducing power of the extract was determined by the method of Oyaizu (1986) [15].

Statistical analysis

Tests were carried out in triplicate for 3–5 separate experiments. The amount of extract needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically estimated using a nonlinear regression algorithm.

Results and Discussion

Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans. Plant produces these chemicals to protect itself but recent research demonstrates that many phytochemicals can protect humans against diseases. There are many phytochemicals fruits and herbs and each works differently. In the present study, phytochemical screening of *Commelina maculata* leaf extract showed the presence of polyphenol, flavonoids, terpenoids, steroids, tannin, saponins, glycosides, phlopatannins, triterpenoids, alkaloids, coumarins, anthroquinones and anthocyanins in ethanol and aqueous extract while alkaloids absent in aqueous extract.

DPPH Assay

DPPH radical scavenging activity of *Commelina maculata* extract and standard as ascorbic acid are presented in Fig 1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nuutila *et al.*, 2003) [16]. Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole.

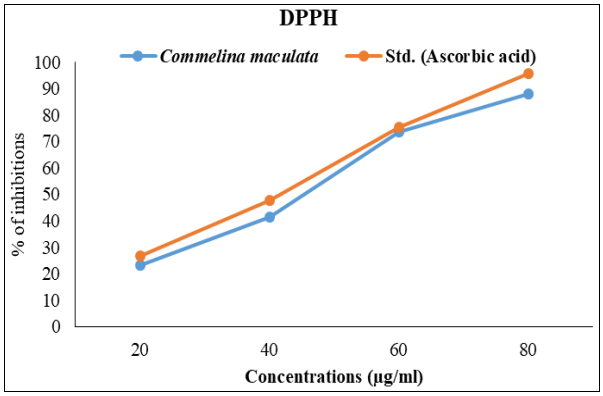

Fig 1: DPPH radical scavenging activity of *Commelina maculata*

The proton transfer reaction of the DPPH• free radical by a scavenger (A-H) causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006) [17]. The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 44.44µg ml^{-1} and 40.39µg ml^{-1} respectively**.** The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay activity is near to standard as ascorbic acid.

Total antioxidant activity

The yield of the methanol extract of the plant extract and its total antioxidant capacity are given in Fig. 2. Total antioxidant capacity of *Commelina maculata* extract is expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al.*, 1999) [11]. Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract. The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 43.27µg ml^{-1} and 40.40µg ml^{-1} respectively**.**

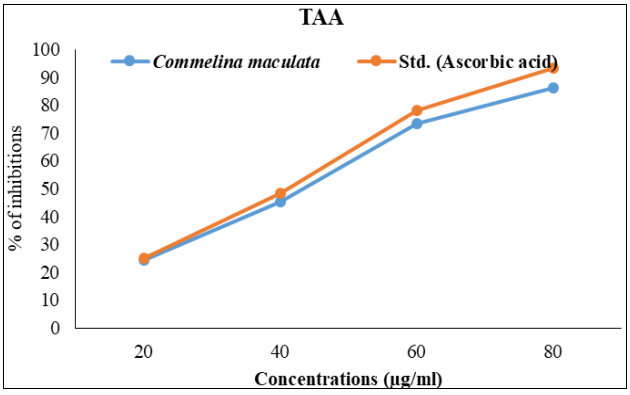

Fig 2: Total antioxidant assay of *Commelina maculate*

Nitric Oxide Scavenging activity assay

Nitric oxide (NO^0) released from sodium nitro prusside (SNP) has a strong NO^+character which can alter the structure and function of many cellular components. The extract of *Commelina maculata* exhibited good NO scavenging activity leading to the reduction of the nitrite concentration in the assay mediam. The NO scavenging capacity was concentration dependent. *Commelina maculata* in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of NO liberated from SNP in the reaction medium with time compared to the standard ascorbic acid. The toxicity of NO increase when it reacts with superoxide to form the peroxynitrite anion (ONOO-) which is a potential strong oxidant that can decompose to produce OH and NO_2 Pacher *et al.* (2007) [18]. The present study shows that *Commelina maculata* increased with increasing concentration (fig 3). The half inhibition concentration (IC_{50}) of plant extract and ascorbic acid were 50.73µg/ml and 41.05µg/ml respectively.

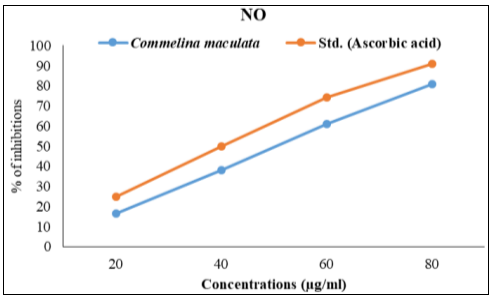

Fig 3: Nitric Oxide Scavenging activity assay of *Commelina maculata*

Hydroxyl radical scavenging activity

Hydroxyl radical is the familiar reactive oxygen species and can causes severe damage to adjacent biomolecules including carbohydrate, lipids, protein and DNA. Hydroxyl radical scavenging activity of ethanolic extract was measured by 1, 10 phenanthroline-Fe^{2+} complex oxidation method. Fe^{2+} was formed when ferrous sulphate added to hydrogen peroxide. This formed ferrous ion reacts with 1, 10 phenanthroline and forms 1, 10 phenanthroline–Fe^{2+} complex which is acts as indicator in oxidation reduction reaction. The hydroxyl radical also formed from the H_2O_2-Fe2+ reaction mixture oxidize from Phenanthroline–Fe^{2+} into Phenanthroline–Fe^{3+} complex. Presence of free radical scavenger in the extract reduces the oxidation reaction accompanied with reduction in the absorbance which can be measured quantitatively at 560 nm (Olabinri *et al*., 2010) [19]. Hydroxyl radical scavenging activity of *Commelina maculata* increased with increasing dosage (Fig. 4). The half inhibition concentration (IC_{50}) of *Commelina maculata* extract and ascorbic acid were 45.15µg/ml and 40.48µg/ml respectively.

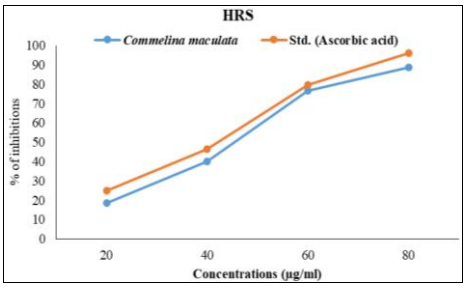

Fig 4: Hydroxyl radical scavenging activity assay of *Commelina maculata*

Reducing power activity

For the measurements of the reducing ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of *Commelina maculata*. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging (Diplock, 1997; Yildirim *et al*, 2000) [20, 21]. Fig. 5 depicts the reductive effect of *Commelina maculata*. Similar to the antioxidant activity, the reducing power of *Commelina maculata* increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that *Commelina maculata* consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

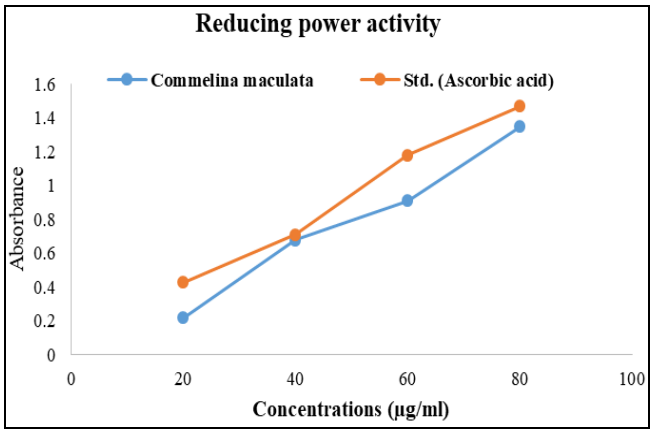

Fig 5: Reducing power assay of *Commelina maculata*

Conclusion

The results of the present study showed that the extract of *Commelina maculata* extract which contains of flavonoids and polyohenols. These phytochemicals are exhibited the greatest antioxidant activity DPPH scavenging, superoxide anion radical scavenging, total antioxidant, metal chelation and iron reducing power activity which participate in various pathophysiology of diseases including cancer, diabetic, ageing etc. This work has gathered experimental evidence on the *Commelina maculata* extract as natural antioxidant for its capacity to scavenge reactive oxygen and nitrogen species and protect cells/organism from oxidative damage and thus could be an effective against oxidative stress. In addition, the *Commelina maculata* extract found to contain a noticeable amount of total phenols which plays a major role in controlling antioxidants. Thus, it can be concluded that *Commelina maculata* extract can be used as an accessible source of natural antioxidants with consequent health benefits.

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