



Research Article

ANTI-OXIDANT ACTIVITY OF *MIRABIDIS JALAPA* AND *CURCULIGO ORCHIOIDES*

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ABSTRACT

Antioxidants are helpful in the defense mechanism of body against different pathogens. The use of plant derived antioxidants is helpful against many degenerative diseases like Parkinson, Alzheimer and Cancer. This evaluation gives an overlook to the different methods used to determine antioxidant capacity of different antioxidants. The explanation of analytical performances and principles of different methods used for the determination of antioxidant capacity involve techniques are discussed in detailed.

Keywords: Antioxidants, DPPH, *Mirabilis jalapa*, *Curculigo orchioides*.

INTRODUCTION

Medicinal plants have been known for their therapeutic potentialities and beneficial impact on health such as antioxidant, anti-inflammatory, antimicrobial and anticarcinogenic effects. These properties are mainly due to phenolic compounds such as polyphenols and flavonoids which have recently received increasing attention (Oueslati *et al.*, 2012). *Mirabilis jalapa* is one of these medicinal plants used for centuries for the treatment of various ailments; its leaves may be eaten cooked as well, but only as an emergency food. An edible crimson dye is obtained from the flowers to color jellies and cakes. It is popularly known as four o'clock. It belongs to the family Nyctaginaceae. It is a large, herbaceous plant grown in gardens throughout India and Pakistan (Rozina, 2016). *Curculigo orchioides* Gaertn. Hypoxydiaceae is an important, endangered, medicinal plant popularly known in India as black musali. The rhizome and tuberous roots of the plant have been used extensively in indigenous medicinal practices in India, Pakistan, and China for the treatment of various diseases, including cancer, jaundice, asthma, and diarthrosis (Dhar & Sudarshan, 1968). The juice extracted from the rhizome has also been used as a tonic to overcome impotence. The *C. orchioides* is a small, geophilous, perennial herb with a long cylindrical rhizome. The plant is found near sea level up to an altitude of 2300

m and in particular on moist laterite soil. The active compounds in this plant have been reported to include flavones, glycosides, steroids, saponins, triterpenoids (Brown *et al.*, 1986; Xu *et al.*, 1992). Asia has abundant species of medicinal and aromatic plants and traditional medicines have practiced in Asia since ancient times. India has made use of medicinal plants to cure ailments of thousands of years (Tamizhazhagan *et al.*, 2017a). The phytochemical research based on ethno-pharmacological information is generally considered an effective approach to the discovery of new effective agents from plant extracts it is used to identify secondary metabolites (Tamizhazhagan *et al.*, 2017b).

Though, human body possesses many defense mechanisms through antioxidant enzymes and non-enzymatic compounds against these oxidative stresses. But when these free radicals go out of control, the organism becomes incapable to scavenge all ROS which may lead to the development of chronic diseases, such as cancer, arteriosclerosis, nephritis, diabetes mellitus, liver injury, rheumatism, ischemia, cardiovascular and neurodegenerative disorders such as Alzheimer's and Parkinson's disease (Al-Nbaheen *et al.*, 2013). The production, processing, and marketing of agricultural goods are central to food security and economic growth. Products derived from ethonobotanical (Tamizhazhagan &

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Pugazhendy, 2017). Conventionally, the plant propagates through seeds and grows only during rainy season. Poor seed setting and germination restrict its abundance in nature, and overexploitation has led to the plant's current endangered status (Augustine & Dsouza, 1997).

MATERIALS AND METHODS

Preparation of extracts

The plant was collected from Department of Botany, Govt. Arts College, Dharmapuri. The plant extracts were prepared as described earlier (Ahmad & Aqil, 2007) with little modification. Hundred (100) grams of dry plant powder were soaked in 1 litre of 97% methanol for 3-5 days with intermittent shaking. At the end of extraction, it was passed through Whatman filter paper No. 1 (Whatman Ltd., England). This methanolic filtrate was concentrated under reduced pressure on a rotary evaporator at 40 °C and then stored at 4°C for further use. The filtrate was reconstituted in a known amount of DMSO to obtain methanol extract of known concentration (Tamizhazhagan *et al.*, 2017).

Antioxidant assay

The antioxidant activity of the plant extracts was tested using two methods: ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. The FTC method was used to measure the amount of peroxide at the beginning of the lipid peroxidation, in which peroxide reacts with ferrous chloride and form ferric ion. The ferric ion then combines with ammonium thiocyanate and produce ferric thiocyanate. The substance is red in colour. The thicker the colour was the higher the absorbance. Whereas the TBA methods measure free radicals present after peroxide oxidation.

DPPH radical scavenging activity

This assay is performed following the method described by Dal & Sahu, (2012). 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) solution (0.1mM) was prepared in methanol by dissolving 1.9mg of DPPH in methanol. The solution was kept in darkness for 30 minutes to complete the reaction. The free radical scavenging activity of the enzymatic plant extracts was determined by DPPH. This antioxidant activity was measured by following the method ; 1ml of 0.1mM methanolic DPPH solution was added to 3ml of enzymatic extracts, at different concentration (0.5, 1.0, 1.5, 2.0 and 2.5g/ml). The mixture was vigorously shaken and left to stand for 30 minutes under subdued light. The absorbance was measured at 517 nm in a UV spectrophotometer. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ascorbic acid, which is a good antioxidant, was taken as a standard in this study. The DPPH radical scavenging activity was calculated by using the following equation.

$$\text{DPPH Scavenging activity (\%)} = (1 - A_s/A_c) \times 100 \text{ mg}$$

Antioxidant activity (DPPH Assay) of different solvent extracts of *M. jalapa*

Antioxidant activity of different solvent extracts such as acetone, chloroform, and methanol extracts of *M. jalapa* was assessed by DPPH assay and the data pertaining to the above experiments are shown tables respectively. The acetone extract of *M. jalapa* showed the IC₅₀ concentration of 65mg/ml., whereas, 45 and 40 mg/ml. concentration were recorded as IC₅₀ values against the ethyl acetate and methanol extracts of *M. jalapa*. Various percentages of antioxidant activities noted in to the above experiment.

Antioxidant activity of different solvent extracts of *C. orchoides* and *M. jalapa*

The antioxidant activity of hexane, ethyl acetate and methanol extract was evaluated by the green phosphomolybdenum complex formation as prescribed in the methodology. The absorbance of mixture was measured in 695 nm. This assay has been routinely used to evaluate the antioxidant capacity of the extracts. Various extracts of *C. orchoides* and *M. jalapa* were assessed to determine their antioxidant capacities by the formation of green Phosphomolybdenum complex. The formation of the complex was measured by the intensity of absorbance in extracts at a concentration of 100mg/ml at 95°C as shown in table. This method is based on the reduction on the reduction of Mo (V) by the antioxidant compounds.

RESULT AND DISCUSSION

Antioxidant activity of different solvent extracts such as methanol extract of *C. orchoides* was assessed by DPPH assay and the data pertaining to the above experiments are shown tables respectively. The acetone extract of *C. orchoides* showed the IC₅₀ concentration of IC₉₀ mg/ml, whereas, 63 and 40 mg/ml. concentration were recorded as IC₅₀ values against the ethyl acetate and methanol extracts of *C. orchoides*. The total antioxidant level was highly presented in the plants extracts compared with other standard solutions fractions were analyzed three have good activity against standard BHT (Table 1). The DPPH free radical compound has been widely used to test the free radical scavenging ability of various food samples; the antioxidant present neutralizes the DPPH by the transfer of an electron or hydrogen atom. The reduction capacity of DPPH could be determined by colour changes from purple to yellow by read at 517 nm (Table 1). The methanolic extract of *C. orchoides* and *M. jalapa* demonstrated H-donor activity in our study. The DPPH radical scavenging activity of extracted material was detected and compared with standard antioxidant - vitamin C. The extract of *C. orchoides* and *M. jalapa* tested against DPPH stable radicals spectrophotometrically which reveals that the radical scavenging activity of *C. orchoides* and *M. jalapa* methanol extract possessed excellent antioxidant capacity by increased with the increasing concentration of the extract (Table 2). At a concentration of 100 µg/ml of methanol extract the percentage of inhibition was found to

be 78%. However, the scavenging activity of ascorbic acid at the same concentration was 44.67%. The methanol extract of *C. orchoides* and *M. jalapa* was found at the concentration of 50.50 µg/ml. The methanolic extract of *C. orchoides* and *M. jalapa* effectively reduced the generation of nitric oxide from sodium nitroprusside. *C. orchoides* and *M. jalapa* methanol extract showed oxide scavenging activity at the concentration of 48.67 and 45.67 while the standard control was showed 48.67 and 45.67µg/ml (Table 2). The data clearly revealed that 4.563 and 3.71 AAE/100g

extracts were recorded against hexane extract of *C. orchoides* and *M. jalapa* respectively. Similarly 46.816 and 52.676AAE/100g extracts were recorded in ethyl acetate extract of *C. orchoides* and *M. jalapa*. The maximum antioxidant activity 64.169 AAE/100g was recorded in methanol extract and found significant among the three solvent extracts tested in *C.orchoides*. Similarly, 65.577AAE/100g extracts was noted as the highest antioxidant activity in methanol extract of *M. jalapa* and found statistically significant.

Table1. Antioxidant activity (DPPH Assay) of methanol extract of *Mirabilis jalapa*

Concentrations tested µg/ml	OD value	% activity
20	0.332	44.67
40	0.326	45.67
60	0.311	48.17
80	0.304	49.33
100	0.297	50.50
120	0.281	53.17
140	0.273	54.50
160	0.265	55.83
180	0.249	58.50
200	0.235	60.83
Control	0.6	00

Table 2. Antioxidant activity (DPPH Assay) of methanol extract of *Curculigo orchoides*

Concentrations tested µg/ml	O.D value	% activity
20	0.348	42
40	0.342	43
60	0.41	31.67
80	0.332	44.67
100	0.326	45.67
120	0.311	48.17
140	0.308	48.67
160	0.301	49.83
180	0.241	59.83
200	0.211	64.83
Control	0.6	00

Several techniques have been used to determine the antioxidant activity *in vitro* in order to allow rapid screening of substances since substances that have low antioxidant activity *in vitro*, will probably show little activity *in vivo* (Appeltans *et al.*, 2012). Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms (Umamaheswari & Chatterjee, 2008). The electron donation ability of natural products can be measured by 2, 20-diphenyl-1- picrylhydrazyl radical (DPPH) purple-coloured solution bleaching (Appeltans *et al.*, 2012). The

method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test (Krishnaiah *et al.*, 2011).

In the present study among all the extracts tested methanol showed significantly higher inhibition percentage and positively correlated with total phenolic content. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating

hydrogen to a free radical to scavenge the potential damage. Superoxide radical is considered a major biological source of reactive oxygen species (Khachatryan *et al.*, 2010). Although superoxide anion is a weak oxidant, it gives rise to generation of powerful and dangerous hydroxyl radicals as well as singlet oxygen, both of which contribute to oxidative stress (Meyer & Isaksen, 1995). The antioxidant capacity of the fractions was measured spectrophotometrically through phosphomolybdenum method, based on the reduction of Mo (VI) to Mo (V) by the test sample and the subsequent formation of green phosphate/Mo (V) compounds. The present study demonstrated that MLN exhibited the highest antioxidant capacity for phosphomolybdate reduction. Recent studies have shown that many flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants (Sharififar *et al.*, 2009; Trinh *et al.*, 2012).

CONCLUSION

In addition to those pharmaceutical properties of *M. jalapa* reported in the literature, this research showed that leaves of this plant may possess considerable antioxidant activities compared to the rest of the medicinal plants as well as BHA and ascorbic acid (as positive controls). Thus, further research may be warranted to study active compounds of *M. jalapa* that confer the antioxidant activity. The findings presented here might have implications in the population disease prevention field, newer, safer; easy consume common peoples there are no side effects.

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