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EVALUATION OF IN-VITRO FREE RADICAL SCAVENGING ACTIVITY OF *MORINGA OLEIFERA LEAF* EXTRACT USING FENTON REACTION

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ABSTRACT
Moringa oleifera Linn, the most widely cultivated species of a monogeneric family Moringaceae. Moringa oleifera is one of the 14 species of family Moringaceae, native to India, Africa, Arabia, Southeast Asia, South America, the Pacific and Caribbean Islands. The present investigation deals with the evaluation of antioxidant activity of the leaves of Moringa oleifera using Fenton Reaction. The dried leaves of Moringa oleifera was extracted with methanol using a Soxhlet extractor. The total phenolics content of leaf as determined by Fenton reaction and was found to be good antioxidant activity as different dose concentrations. The antioxidant activity of plant extract was carried out with ascorbic acid as a standard reducing agent. The present results were made with the use of UV-Visible Spectrophotometer. In this plant Moringa oleifera leaf Extract there was a remarkable concentration dependent free radical scavenging and reducing power was exhibited. In conclusion the present study indicates that Moringa oleifera leaf extract may be a potential source of natural antioxidant.

KEY WORDS: Moringa oleifera; extraction; hydroalcoholic extract; phenolic content; flavonoid content; antioxidant activity.

1. INTRODUCTION

At the present point in time the modern conventional healthcare is hampered with great problems of unsafe medicines, chronic diseases, resistant infections, autoimmune disorders and degenerative disorders of ageing, even though great scientific advances. More than 70 % of India s 1.1 billion populations still use these non- allopathic systems of medicines (Paul et al., 2006). Medicinal plants and derived medicines are widely used in traditional cultures all over the world and they are becoming increasingly popular in modern society as natural alternatives to synthetic chemicals (Vanwyk, et al., 2009). In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects (Patel et al., 2001). The World Health Organization (WHO) estimated that approximately 80 % of world population relies mainly on traditional medicines, mostly plant drugs in their health care (Priyanka et al., 2013).

Flowers have been used for edible purpose since ancient times, and have medicinal as well as nutritional value. Now a day, in the Western world, the most common use of flowers is in salads. But more and more people are becoming adventurous as they realize the flavor and health potential of flower blossoms and bud (Sharma et al.2011). Edible flower is just what the name implies, a flower or part of flower that can be eaten. Although edible flowers are most popular in fresh salads and imaginative uses for the colored petals are being explored that open beautiful and tasty culinary vistas (Mlcek, 2011). Edible flowers becoming more popular as evidenced by an increase in the number of edible flower cook books, culinary magazine articles, and television shows. Consumer purchase packaged edible flowers for use in meals as a garnish or ingredients in salads, soups, desserts and drinks (Pegoraro, 2007). Moringa oleifera is the most widely cultivated species of a monogeneric family, the Moringaceae, that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. This rapidly- growingtree (also known as the horse radish tree, drumstick tree, benzolive tree, kelor, marango, mlonge, moonga, mulangay, nébéday, saijhan, sajna or Ben oil tree),was utilized by the ancient Romans, Greeks and Egyptians;it is now widely cultivated and has become naturalized in many locations in thetropics (Fahey,2005).Described as one of the most amazing trees God has created, almost every part of drumstick viz. bark, root, fruit, flowers, leaves, seed and gum is a rich repository of proteins, vitamins and minerals including potassium, calcium, phosphorus, iron,

folic acid as well as carotene. Leaves can be eaten fresh, cooked or stored as dry powder for many months without refrigeration, without loss of nutritional value. Almost all the parts of this plant have been used for various ailments in the indigenous medicine of South Asia (Price, 1985; Parrotta, 2001). Almost all the parts of this plant: root, bark, gum, leaf, fruit [pods], flowers, seed and seed oil have been used for various ailments in the indigenous medicine of South Asia, including the treatment of inflammation and infectious diseases along with cardiovascular, gastrointestinal ,hematological and hepatorenal disorders (Kumar et al., 2010). The flowers can be eaten or used to make a tea. In Haiti, tea from the flowers is drunk for colds. The flowers provide good amounts of calcium and potassium. Moringa flowers also provide a year-round source of nectar for bees, although some have claimed that honeybees do not gather nectar from Moringa (Price, 2007).Different parts of this plant including flower are used in folk medicine to cure various diseases. Leaves are used as antiseptic and in kidney troubles, muscular pain, piles and applied to boils and carbuncles. The flower is useful in fevers, epileptic fits (Ayurveda), astringent, carminative, stomachic, scabies and liver complaints and is also employed in diseases of the eyes (Nikkon et al., 2009). Therefore, this current article ended up being undertaken exclusively to examine the role connected with Methanol extract of M. oleifera Linn leaves as a potential anti-oxidant agent.

2. MATERIALS AND METHODS

Plant material – *Moringa oleifera* leaf was collected from Local Herbal Garden, Raipur (Chhattisgarh), India.

Chemicals and Reagent samples – The reagents used were of highest purity (>99.95%) and were purchased from Sigma Chemical Co. (Germany) and other. Sample absorbances were read using a Lambda 532 nm, UV Spectrometer made by Varian.

Preparation of extract - Dried powdered of *Moringa oleifera* leaf (15 g) were extracted by continuous mixing in 500 ml 50% methanol and water, 24 h at room temperature. After the filtration process, methanol was evaporated until only water remained through evaporation on water bath at 60-70 0c temperature. The final extract was kept in air tied box.

Deoxyribose assay to assess OH -radical scavenging activity

The OH- radical scavenging activity of *Moringa oleifera* leaf extract (10–100 ug/ml) was determined according to the deoxyribose method reported of Halliwell, *et al*., (1987). In the protocol the presence of 100 lM EDTA. $FeCl_3$, H_2O and ascorbic acid were prepared in degassed H_2O prior to use. The reaction tube contained (final concentrations) 3.6 mM deoxyribose, 100 lM EDTA, 1 mM H2O2, 100 lM L- ascorbic acid, 100 lM $FeCl_3$, H_2O in 25 mM phosphate buffer, pH 7.4 in 1.0 ml total volume. Samples was kept in incubation at 38º C, 1 hrs, 1.0 ml 1.0% TBA in 0.05 M NaOH and 1.0 ml 10% TCA were added to the reaction mixture after that samples was heated in a boiling water bath for 15 min. After the samples were cooled, the absorbance's were read at 532 nm. The IC50 value of the plant extract was compared with that of ascorbic acid, which was used as the standard. The result of lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The percentage of inhibition of hydroxyl radical was calculated as follows:

% Inhibition =

Abs:$\frac{\text{532 nm Control Abs. - 532 nm sample Abs.} \times 100}{\text{532 nm Control Abs}}$

Antioxidant capacity of test compounds was expressed as IC_{50}, the concentration necessary for 50% inhibition concentration of TBARS.

3. RESULT

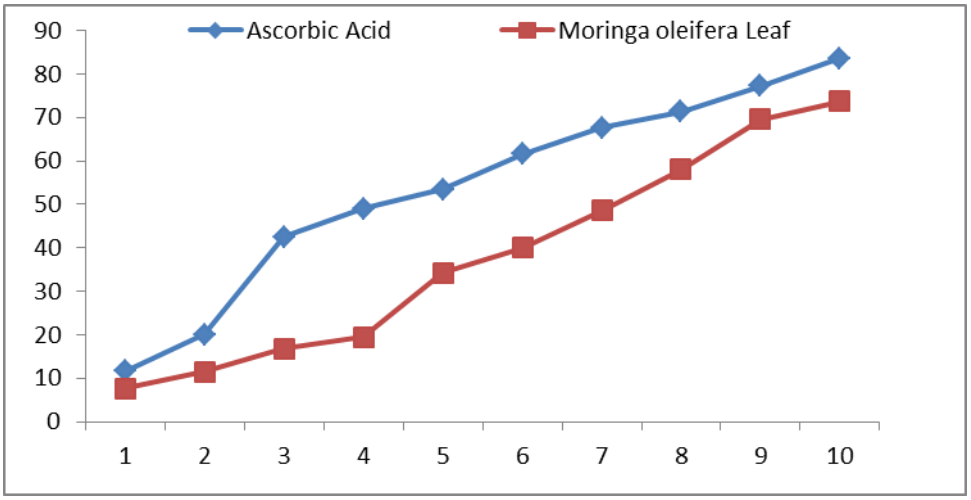
The results of the effects of the examined *Moringa oleifera* leaf extract as well as control solutions on OH-radical production. They show that all extract of *Moringa oleifera* leaf *extract* and control solutions as a ascorbic acid inhibited the production of OH- radicals. The % of free racial scavenging activity of hydro-methanolic extract of *Moringa oleifera* leaf presented in Table 1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

Table 1: Antioxidant activities of *Moringa oleifera* leaf extract using Fenton reaction.

Concentration (in µl)	Ascorbic acid (Mean +SE)	*Moringa oleifera* Leaf (Mean + SE)
10	11.67 ± 1.38	7.57 ± 0.89
20	20.02 ± 1.27	11.47 ± 1.23
30	42.48 ± 1.08	16.74 ± 1.27
40	49.02 ± 0.92	19.48 ± 1.25
50	53.42 ± 2.53	34.25 ± 1.00
60	61.65 ± 2.72	40.04 ± 0.53
70	67.67 ± 1.92	48.61 ± 0.84
80	71.28 ± 1.16	58.10 ± 0.82
90	77.18 ± 0.86	69.49 ± 0.73
100	83.63 ± 0.73	73.72 ± 0.18

IC_{50} Values

Sl	Group	IC_{50} Value
1.	Ascorbic acid	43.0 (µg/ml)
2.	Moringa oleifera Leaf	73.0 (µg/ml)



Graph 1: Antioxidant activities of Ascorbic acid and Beta *vulgaris* root extract using Fenton reaction.

4. DISCUSSION

The Indian medicinal system remains the most ancient yet living traditional with sound philosophical and experimental basis. It is known to be complete medical system that comprised physical, physiological, philosophical, ethical, and spiritual health (Semwal, et al., 2015). In countries beyond India, Ayurveda therapies and practices have been integrated in general wellness applications and in some cases in medical use (Populorum and Michael Alexander, 2008).

The natural and medicinal plants are able to produce a large number of diverse bioactive compounds. High concentrations of phytochemicals, which may protect against free radical damage, accumulate in fruits and vegetables (Suffredini, et al., 2004). Many of plants metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. In the worldwide it is proved that plants produce these chemicals to protect themselves, but recent researches demonstrate that many phytochemicals can also protect human against diseases (Narasinga Rao, 2003).

Antioxidant from natural source can improve the antioxidant system in body for scavenging free radicals. An interest in antioxidant from natural sources increasing faster than synthetic sources. Phenolic compounds which naturally present in M. oleifera plant can reduce the risk of many diseases and its effects which correlated with the antioxidant compounds. Recently, there are some reports about M. oleifera leaves which rich in phenolic compounds such as flavonoids, gallic acid, quercetin and kaempferol as antioxidant activity (Santos, et. al., 2012). M. oleifera is a one of Indonesian traditional plant that has multipurpose biological activities.

In our study the effects of the examined *Moringa oleifera* leaf extract as well as control solutions on OH- radical production. They show that all extract of *Moringa oleifera* leaf *extract* and control solutions as a ascorbic acid inhibited the production of OH- radicals. The % of free racial scavenging activity of hydro-methanolic extract of *Moringa oleifera* leaf presented in Table 1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

The hydroalcoholic extract of Moringa oleifera exhibited good antioxidant result. This could be attributed due to presence of phytoconstituents such as polyphenolic compounds (flavonoids). Plant extract showed strong antioxidant capacity in vitro and the extract can be considered a good source of natural antioxidant.

5. CONCLUSION

Plants are responsible for preventing disease and promoting health have been studied extensively to establish their efficacy and to understand the underlying mechanism of their action. Plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack (Gibson, et al., 1998; Mathai, 2000). Many of advance research clearly proved that they have roles in the protection of human health, when their dietary intake is significant.

Antioxidant molecule inhibits the oxidation of other molecules from the body. Natural and artificial oxidation is a chemical reaction that can produce free radicals,

leading to chain reactions that may damage cells. Antioxidants such as thiol or ascorbic acid (vitamin C) terminate these chain reactions. The natural and synthetic antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as Cancer or coronary heart disease. The hypothesis that antioxidant supplements might promote health has not been confirmed experimentally (Abner, et al, 2011).

In our test system the effects of the examined *Moringa oleifera* leaf extract as well as control solutions on OH-radical production. They show that all extract of *Moringa oleifera* leaf *extract* and control solutions as a ascorbic acid inhibited the production of OH- radicals. The % of free racial scavenging activity of hydro-methanolic extract of *Moringa oleifera* leaf presented in Table 1 have reducing power, the free radial OH- scavenging activity of the extract increases with increasing the concentration.

This finding provides scientific evidence for the Indian traditional people way, which used M. oleifera leaves as one of nutrition food to prevent diseases. This study also indicated that M. oleifera leaves can be used as antioxidant source.

In the present study, the phenolic content of *Moringa oleifera* leaf was found to be high which might have responsible for its antioxidant and free radical scavenging activity in the in vitro study models. *Moringa oleifera* leaf extract can be used as a beneficial medicinal herb for reducing the free radicals present in body due to high levels of polyphenolic antioxidant compounds.

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