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Antioxidant Activity of *Moringa oleifera* using Successive Solvent Extraction Technique

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ABSTRACT: *Moringa oleifera* Lam (syn. *Moringa pterygosperma*), 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 different solvents (petroleum ether, chloroform, ethyl acetate and 70% ethanol) by successive solvent extraction method. DPPH quenching ability of *Moringa oleifera* extract was measured. The phenolic content was found more in comparison to flavonoid content in hydoalcoholic extract (70% ethanol-water extract) . The antiradical activity was expressed as IC_{50} (µg/mL), the antiradical dose required to cause a 50% inhibition. Vitamin C was used as standard. The overall results showed that the hydroalcoholic extracts of leaf of *Moringa oleifera* showed potent antioxidant activity.

Key Words: *Moringa oleifera;* successive solvent extraction; hydroalcoholic extract; phenolic content; flavonoid content; antioxidant activity.

I. INTRODUCTION

Moringa oleifera is the most widely cultivated species of family Moringaceae. It has been given several names including , horseradish tree, benzoil tree, kelor, marango, mlonge, moonga, saijhan, sajna and ben oil tree. It is a rapidly-growing tree utilized worldwide. Moringa oleifera is considered one of the world's most useful trees, as almost every part of the tree has some medicinal, nutritional and other beneficial properties. In Indian subcontinent this tree have been using as a regular component of conventional eatables for nearly 5000 years. *Moringa oleifera* tree grows well in the humid or hot dry land with average height of 5 to 10 m. It can survive in harsh climate as well as drought resistant condition. Plant extracts are some of the most attractive sources of new drugs, and have been shown to produce promising results for the treatment of many ailments like diabetes, arthritis and ulcers.The medicinal properties have been attributed to phytochemical compositions of various parts of *Moringa oleifera* ; the roots, bark, leaf, flowers, fruits, and seeds [8-9]. *Moringa oleifera* is one such plant that has been identified to contain good antioxidants property [6-7]. The dry leaves of *Moringa oleifera* is widely utilized in the developing countries as a good source of calcium and protein. The leaves, young shoots, are eaten in vegetable curries, as pickles and as salads. The leaf can be eaten fresh and cooked, or as reported that if leaves are stored as dry powder for many months without refrigeration then there is no loss of any nutritional value.

The Moringa plant, found in tropical and subtropical countries, provides a rich and rare combination of zeatin, quercetin, kaempferom and many other phytochemicals. It is very important for its medicinal value. Various parts of the plant such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumour, antipyretic, antiepileptic, anti-inflammatory and antiulcer(Pal *et al*, 1995). To date, a variety of biological activities of parts of *M. oleifera* have been reported. Therefore, this current article ended up being undertaken exclusively to examine the role connected with aqueous, chloroform and petroleum ether extracts of *M. oleifera* Lam. leaves as a potential anti-oxidant agent.

II. EXPERIMENTAL SECTION

The Leaf of plant *Moringa oleifera* was collected from local area of Sagar (M.P.), India in the month of June. The plant specimen was confirmed and given herbarium number Bot/Her/1013 by Dr. Pradeep Tiwari, Dept. of Botany, H.S. Gour University, Sagar (M.P).

The leaves of selected plant species were separated carefully, cleaned, shade dried, mechanically grinded and a coarse powder was prepared. Dried powdered Leaf of plant *Moringa oleifera* has been successively extracted with petroleum ether, chloroform, ethyl acetate and hydroalcoholic (Ethanol 70%) using soxhlets apparatus for 48 hrs, filtered and dried using vaccum evaporator at 40°C. (Tiwari *et al*., 2011). Phytochemical screening was carried out in the extract with highest percentage yield using commonly employed chemical reactions.The chemical tests were performed for testing different chemical groups present in extracts.

Total Phenolic content estimation

Principal: The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method.

Preparation of Standard: 50 mg Gallic acid was dissolved in 50 mL methanol, various aliquots of 25-150µg/mL was prepared in methanol.

Preparation of Extract: 1gm of dried powder of drug was extracted with 100 mL methanol. Then filtered and make up the volume up to 100 mL. 1 mL (1mg/mL) of this extract was for the estimation of flavonoids.

Procedure: 1 mL of extract or standard was mixed with 5 mL of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 mL (75g/L) of sodium carbonate. The mixture was vortexed for 15sec and allowed to stand for 30min at 40°C for colour development. The absorbance was measured at 765 nm using a spectrophotometer.

Total flavonoids content estimation

Principal: Determination of total flavonoids content was based on aluminium chloride method.

Preparation of standard: 50 mg quercetin was dissolved in 50 mL methanol, and various aliquots of 25- 150µg/mL were prepared in methanol.

Preparation of extract: 1gm of dried powder of drug was extracted with 100 mL methanol. Then filter and make up the volume up to 100 mL. 1ml (1mg/ml) of this extract was for the estimation of flavonoid.

Procedure: 1 mL of 2% $AlCl_3$ methanolic solution was added to 1 mL of extract or standard and allowed to stand for 60 min at room temperature, and then absorbance was measured at 420 nm.

Antioxidant activity

DPPH reducing power assay

DPPH quenching ability of *Moringa oleifera* extract was measured. Methanolic solution, DPPH solution (0.01M) was mixed with serial dilutions (20–200 µg/mL) of the extract and after 20 min, the absorbance was read at 517 nm. The antiradical activity was expressed as IC_{50} (µg/mL), the antiradical dose required to cause a 50% inhibition. Vitamin C was used as standard. The ability to scavenge the DPPH radical was calculated using the following equation:

DPPH scavenging effect (%) = $(A_0\text{-}A_t/ A_0) \times 100$

Where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. All samples were analyzed in triplicate.

III. RESULTS AND DISCUSSION

From the results obtained it is clear that the *Moringa oleifera* plant shows the presence of alkaloids, glycosides, saponins, tannins, flavonoids, amino acid and terpenoids in leaf parts when extracted with different solvents using soxhlet extraction procedure. The phytochemical analysis of hydroalcoholic leaves extract of *Moringa oleifera* plant indicates the presence of alkaloids, steroids and flavonoids in sufficient quantity according to preliminary phytochemical analysis. Phenolic and flavonoids are the phytochemicals that are present in hydroalcoholic extract.

The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve.

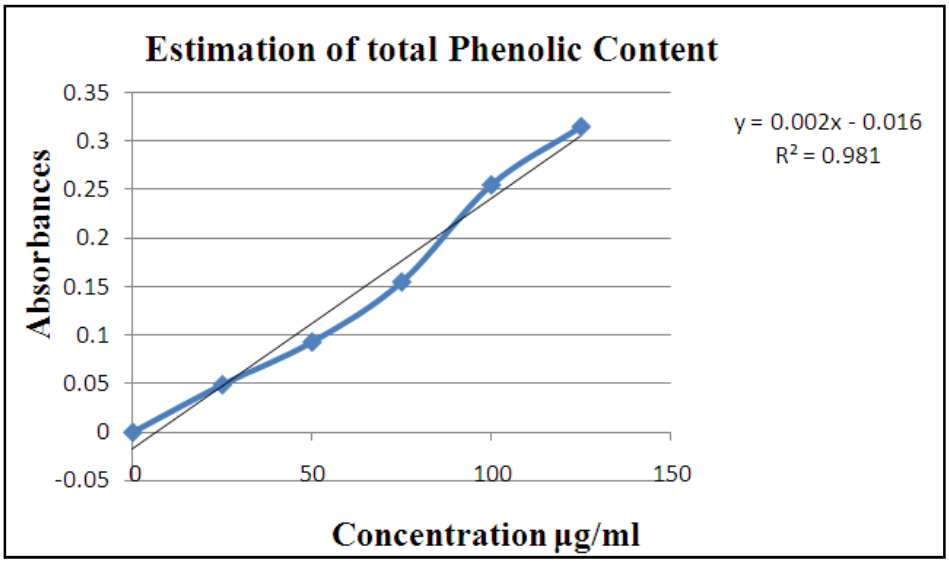
$Y = 0.002X - 0.025$

$R^2 = 0.980$

where X is the absorbance and Y is the Gallic acid equivalent (GAE).

Table 1: Preparation of calibration curve of Gallic acid.

S.No.	Concentration(µg/mL)	Absorbance(nm)
0	0	0
1	25	0.049
2	50	0.093
3	75	0.155
4	100	0.255
5	125	0.315
6	150	0.421



Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation based on the calibration curve.

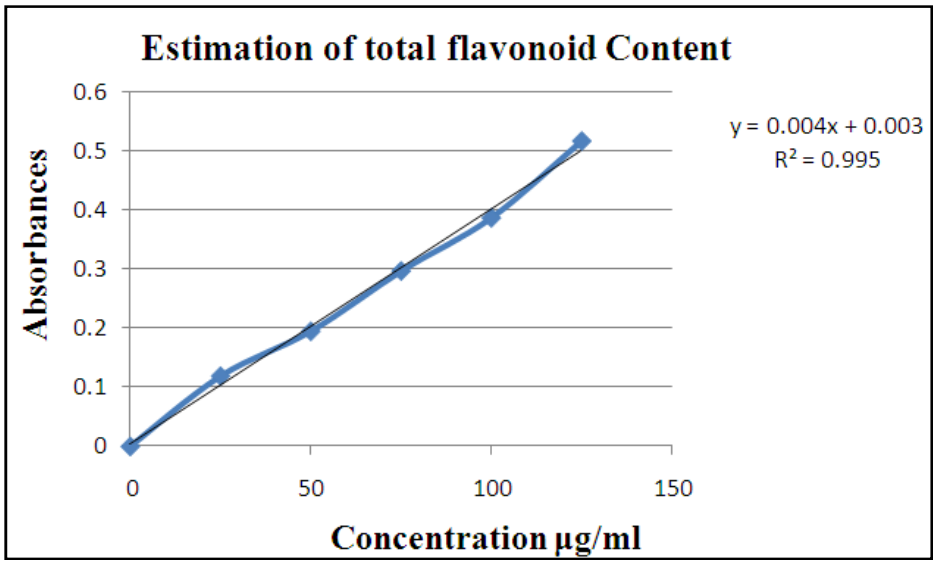
Y = 0.004 X - 0.001

R^2 = 0.996

where X is the absorbance and Y is the quercetin equivalent (QE).

Table 2: Preparation of calibration curve of Quercetin

S.No.	Concentration(µg/mL)	Absorbance(nm)
0	0	0
1	25	0.119
2	50	0.195
3	75	0.297
4	100	0.387
5	125	0.517
6	150	0.626



Graph of Estimation of Total flavonoid content

Table 3: Estimation of total phenolic and total flavonoid content.

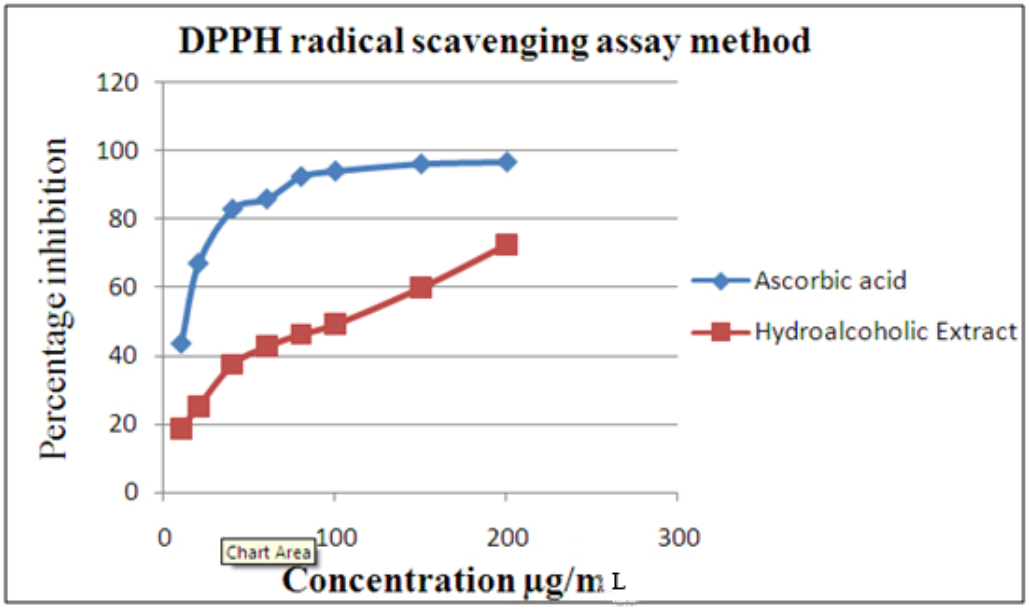
S. No	Extracts	Total phenolic content (mg/100g of dried powder)	Total flavonoid Equivalent to Quercetin mg/ 100 mg of dried extract
1	Hydrolacoholic extract	140.24±0.747	60.43±0.220

The total phenolic and flavonoid content of the hydroalcoholic extract was also determined. In extract phenolic content was found more in comparison to flavonoid contents.

There was a marked fall in maximum absorbance level by DPPH combined with the various concentrations of polyherbal preparation at 517 nm in U.V spectrophotometer.

Table 4: DPPH radical scavenging assay method.

Concentration (µg/mL)	Ascorbic acid	Hydroalcoholic Extract
10	43.43±1.08	18.79±0.46
20	67.02±1.46	25.35±1.23
40	82.97±2.08	37.58±1.06
60	85.99±0.46	42.81±0.96
80	92.55±0.98	46.45±0.32
100	94.14±0.45	49.30±0.65
150	96.27±0.26	60.04±0.86
200	96.82±0.42	72.69±0.42
IC_{50}	**11.69±0.66**	**103.5±1.05**



DPPH radical Scavenging assay of various extracts of *Moringa oleifera.*

The above figure and table shows the comparative data of DPPH radical scavenging activity as determined by the IC_{50} values of the leafy extract. An IC_{50} value is the concentration of the sample required to scavenge 50% of the free radicals present in the system. IC_{50} value is inversely related to the antioxidant activity of crude extracts. *Moringa oleifera* as compared to ascorbic acid found is 103.5±1.05, IC_{50} value.

IV. CONCLUSION

Antioxidant activity was evaluated using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. The antioxidant compounds such as flavonoids, carotenoids and anthocyanidin in the plant extract scavenge free radicals such as superoxide (O_2), hydrogen peroxide (H_2O_2), nitrogen radicals (NO^-) coming from metabolic pathways of human. The hydroalcoholic extract of *Moringa oleifera* exhibited good antioxidant result. This could be attributed due to presence of phytoconstituents such as polyphenolic compounds (flavonoids).

REFERENCES

[1]. Prashant Tiwari, Bimlesh Kumar, Mandeep Kaur, Gurpreet Kaur and Harleen Kaur, (2011). *International Pharmaceutical Sciencia*, **1**(1), 98-106.

[2]. Pal, S.K., Mukherjee, P.K., and Saha, B.P. (1995). Studies on the antiulcer activity of *M. oleifera* leaf extract on gastric ulcer models in rats. *Phytother. Res.*, **9**, pp. 463-465.

[3]. Pillai, N.R., Suganthan, D., Seshari, C., and Santhakumari, G. (1978). Antigastric ulcer activity of nimbidin. *Indian Journal of Medical Research*, **68**, pp.169-175.

[4]. Makonnen, E., Hunde, A., and Damecha, G. (1997). Hypoglycaemic effect of M. stenopetala aqueous extract in rabbits. *Phytother. Res.*, **11**, pp. 147-148.

[5]. Uguzlar Huseyin, Maltas Esra and Yildiz Salih. (2011**).** Antioxidant activity and Fatty acid composition of *Arum dioscoridis* Extracts. *Biosciences, Biotechnology Research Asia* **8**(1), 75-82.

[6]. Al-Bari M, Sayeed MA, Rahman Ms, Mossadik MA (2006) .Characterization and antimicrobial activities of a phenolic acid derivative produced by *Streptomyces bangladeshiensis*, a novel species collected in Bangladesh. *Med Sci* 1,77-81.

[7]. Hertog MG, Sweetnam PM, Fehily AM, Elwood PC, Kromhout D (1997). Antioxidant flavonols and ischemic heart disease in a Welsh population of men: the Caerphilly study. *Am J Clin Nutr* **65**,1489-94.

[8]. Anwar *et al*., (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extract. *Molecule* **14**, 2167-80.

[9]. Dhenge R.M. and Itankar P. R. **(**2008). Antiinflammatory and antioxidant potential ethanolic extract of *Indigofera trifoliate*. *Indian J. Nat. Prod.*, **24**(1), 3.

[10]. Riddhi M Patel *et al*., (2015). *Cibtech Journal of Bio-Protocols*, **4**(1), 46-53.

[11]. Rajanandh MG *et al*. (2010). *International Journal of Pharm Tech Research*, **2**(2), 1409-1414.

[12]. Cheng, Z., Moore, J. and Yu, L. (2006). High-throughput relative DPPH radical scavenging capacity assay. *Journal of Agricultural and Food Chemistry*. **54**(20). 7429–7436.

[13]. Pal S. K., Mukherjee P.K. and Saha B.P. (1995). Studies on antiulcer activity of *Moringa oleifera* leaf extract on gastric ulcer models in rats. *Phytother. Res.* **9**.463-465.

[14]. Sonia Goswami and Rashmi Singhai (2015). Phytochemical analysis of hydroalcoholic extract of leaves of *Moringa oleifera*. *International journal of green and herbal chemistry* **4**(3). 1-10.

[15]. N. Singh *et al*., (2012). Anti ulcer and antioxidant activity of *Moringa oleifera* (Lam) leaves against asprin and ethanol induced gastric ulcer in rats. *Int. Res. J. of Pharmaceuticals* 2(2). 46-57.

[16]. Suphachai Charoensin *et al*., (2014). Antioxidant and anticancer activities of *Moringa oleifera* leaves. *Journal of Medicinal Plant Research* **8**(7). 318-325.

[15]. Dhanarajan. M. S, Rajalakshmi A, Jayachitra A, Pardhasaradhi Mathi, Narasimharao Bhogireddy (2014). Analysis of Phytochemicals, Antibacterial and Antioxidant activities of *Moringa oleifera* Lam. Leaf extract- an in vitro study. *Int. J. Drug Dev. & Res* **6**(4).