International Journal of Universal Science and Technology
ISSN: **ISSN: 2454-7263** Copyright © Universal Print
Volume No. 03, Issue No. 05, Page No. 242-248 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Evaluation of Phytochemical Screening …

Evaluation of Phytochemical Screening and *In – Vitro* Antioxidant Activity of Polar Extracts *Moringa pterygosperma* Flowers

Rohit Kumar Bargah* and Chinmoyee Das

*Department of Chemistry, Govt. S.P.M. College Sitapur, Dist. Surguja (Chhattisgarh) 497111, India
Email:rohitbargah1978@gmail.com, Mobile No -09755387988

Abstract

Moringa pterygosperma is a highly valued plant. The bioactive compounds present in the plant are responsible for the medicinal properties of the plant. The purpose of the study was to evaluate the antioxidant activity of ethanolic and aqueous extract of *Moringa pterygosperma* flowers. The shade dried flowers were extracted with 95% ethanolic and distilled water under sonication. The ethanol and aqueous extracts were screened for phytochemical analysis. Further the ethanol and aqueous extracts were investigated for in vitro antioxidant in different models. The Phytochemical screening revealed the presence of flavonoids, alkaloids, terpenes and triterpenoied, carbohydrate and phenolic compound in ethanol extracts, while saponins, tannins, glycoside were absent in aqueous extract. The polyphenol (61.4 ±0.45 mg GAE/ g extract) and flavonoid content was found to be highest in the ethanol extract (57.4 ±0.17 mg catechin/ g extract). The IC_{50} values of ethanol and aqueous for the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging test was found to be 40.11μg/ml and 57.28 μg/ml, respectively. The results demonstrated that ethanol extract possessed the best antioxidative activity and may be used as a potential source of natural antioxidant against free radical associated diseases.

Keywords: *Moringa pterygosperma,* Phytochemical Screening, Antioxidant, DPPH, Flavonoids

1. Introduction

Natural antioxidants contained in foods from plant origin involving control most of these radicals and so are therefore essential methods inside acquiring as well as preserving a healthy body . *Moringa pterygosperma* Gaertn.(Moringaceae) is a tree growing up to 5-12 M with a open umbrella shaped crown native to India ,Africa, Southeast Asia and the Caribbean Islands[1].The species is cultivated in many tropical and subtropical regions worldwide. It is known by various name as Drumstick tree, miracle tree, Horseradish tree and Mother`s best friends[2]. Moringa pterygosperma is considered a complete food as it has an impressive range of medicinal uses

International Journal of Universal Science and Technology
ISSN: ISSN: 2454-7263 Copyright © Universal Print
Volume No. 03, Issue No. 05, Page No. 242-248 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Evaluation of Phytochemical Screening …

with nutritional value. Its multiple pharmaceutical effects are capitalized as therapeutics remedy for various diseases in traditional medicinal system [3]. This is a dynamics plant with rich medicinal properties [4] .In Ayurvedic system of medicine, its root, root bark, leaves , flowers and pods are reported as antipyretics, abortificient, anthelmintic , diuretics, anti inflammatory, antispasmodic, antibacterial ,antifungal and antioxidants activities [5-8] . Phytochemical analysis revealed the presence of alkaloid, flavonoid, steroids, tannins, saponins and glycosides as major component. The present study is an attempt to explore the antioxidant activity and phytochemical screenig of ethanol and aqueous extract of flower of the plant.

2. Methods and materials

2.1 Plant Material: The fresh sample of flower of *Moringa pterygosperma* plant was collected in the month of Nov.- December 2016 from college campus sitapur, Dist. - surguja (C.G.) . The plant was then identified by professor N. K. Singh, Department of Botany, Govt. Naveen College Sargaon, Dist.- Mungeli. The flower of *Moringa pterygosperma* were collected and separated or then dried under shade drying for 4-5 days. Then the dried flowers are grinded sieved to get nearly fine amorphous powder.

2.2 Preparation of Extracts: The air dried powder 200 gram extracted by soxhlet apparatus with 500 ml of 80% Ethanol and 500 ml of aqueous for 6 hrs. at room temperature . All the extract were concentrated under reduced pressure 45^0C and stored in an air tight container for further study.

2.3 Preliminary Phytochemical Screening: Phytochemical Screening was performed using standard procedure[9-11] .

I. Tests for alkaloids

i. Dragendorff's test: 1 ml of extract. + 1 ml KBr. An orange-red ppt. (alkaloids present).

ii. Mayer's test: 1 ml of extract + 1 ml of KHgI .Whitish yellow or cream colored ppt. (alkaloids present).

II.tests for glycosides

i. Legal's test: (1 ml Ext. + Pyridine + Na_2 [Fe (CN) $_5$NO]). No colour (Glycoside absent).

ii. Baljet's test: (1ml ext. + 1ml $C_6H_2KN_3O_7$) yellow to orange colour (glycoside present).

III. Tests for carbohydrate

i. Benedict's test: (5ml Benedict's reagent. + 1ml extract) Boil 2 min. and cool.(Red ppt., sugars present).

ii. Molisch's test: Extracts in ethanol separately + drops of 20% w/v solution of α naphthol in ethanol (90%). Shake well + add from side of test tube 1 ml of Conc. H_2SO_4 was Reddish violet ring between junction of the layers (carbohydrates present).

IV.tests for steroids

i. Salkowski test: (Extract + $CHCl_3$ + equal volume of conc. H_2SO_4) was added. Bluish red

International Journal of Universal Science and Technology
ISSN: ISSN: 2454-7263 Copyright © Universal Print
Volume No. 03, Issue No. 05, Page No. 242-248 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Evaluation of Phytochemical Screening …

to cherry in $CHCl_3$ and green fluorescence in the acid (steroidal present).

ii. Liebermann-Burchard test: (Extract + 1 ml of acetic anhydride and dissolved) by warming. The contents were cooled and a few drops of conc. H_2SO_4 were from sides of the test tube. (Blue colour) sterols present.

V. Test for proteins

i. Biuret test : 1ml 40% NaOH + 2 drops 1% $CuSO_4$ soln. till a blue color appear + 1ml extract. Pinkish / purple violet color (protein present).

VI. Test for saponins

(i) Extracts boiled with 1 ml of distilled water and shaken. Foam formed (saponins present).

(ii) Extract + 2 ml of DW + sodium carbonate and shake. The Foam formed (saponins present).

VII. Test for tannins

i) Extract + lead acetate solution. White precipitates (tannins present).

VIII. Tests for flavonoids

i. Shinoda test: Test solution + magnesium turnings Conc. HCl drops pink scarlet.

2.4 Quantitative Phytochemical Screening:

2.4.1 Determination of total phenolics

The total amount of phenolic content of plant extract was determined by Folin Ciocalteu method. The standard solutions were prepared by taking 1, 2, 3, 4, 5µl sample from the stock of 100mg/ml and maintain final volume of 1ml. to this 1ml standard solution, 1ml Folin Ciocalteu´s reagent, previously dilute (1:4) was added. To the mixture, 4ml of sodium carbonate (75g/L) and 10 ml of distilled (1:4) were added and mixed well. The mixture was allowed to stand for 2 Hrs. at room temperature. Contents were then centrifuged at 2000g for 5 min and the absorbance of the supernatant was taken at 760 nm. A standard curve was obtained using various concentrations of Gallic acid equivalents (GAE).

2.4.2 Determination of total flavonols

The total flavonols in the plant extract were estimated using the method of Kumaran et,.al. In this method again 1, 2, 3, 4, 5µl samples were taken from stock of 100mg/ml and final volume of 2ml was maintained. To this 2ml $AlCl_3$ ethanol and 3.0 ml (50 g/l) sodium acetate solutions were added to 1 ml standard solution of different concentration. The total flavonoids content were expressed as rutin equivalents.

2.5 DPPH (2, 2- diphenyl -1 piccryl hydrazyl) radical scavenging activity:

2, 2-diphenyl -1 picryl hydrazyl (DPPH) radical scavenging activity was measured according to the method . Extract solution were prepared by dissolving of different dry extract in methanol to produce a solution of 10mg/ml. 600 µM DPPH was dissolved in 300ml methanol and used as stock solution. The plant extract in methanol at various concentration (1, 2, 3, 4 and 5mg) whose final

International Journal of Universal Science and Technology
ISSN: ISSN: 2454-7263 Copyright © Universal Print
Volume No. 03, Issue No. 05, Page No. 242-248 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Evaluation of Phytochemical Screening …

volume was maintained 1ml and were mixed with an aliquot of 2ml of 600μM DPPH solution in methanol and incubated at 25°C for 30 min. Absorbance of the test mixture was read at 517 nm using a spectrophotometer against a DPPH control containing only 1 ml of methanol in place of the extract. All experiment was performed thrice and the results were averaged. Ascorbic acid was used as a standard [12,13] .

$$\text{DPPH scavenging effect} = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Where, $A_{Control}$ and A_{Sample} standard for absorbance of the control and absorbance of tested extract solution respectively.

3. Result and discussion

The presence of medicinally important molecules in a plant is indicative of its medicinal potential. *Moringa pterygosperma* was tasted for presence of alkaloids, flavonoids , polyphenolic compound , steroids, terpenoids, glycosides in ethenolic and aqueous extracts. Saponins, tanins, anthraquinone are absent in both extracts in *Moringa pterygoperma* flowers (Table 1.1) Phytochemical derived from plant products serve as a prototype to develop less toxic and more effective medicine in controlling the growth of micro organism. Flavonoids, alkaloids and phenolic compounds are a major group of compound of act as primary antioxidant. So due to the presence of such secondary metabolites Moringa pterygosperma may have higher medicinal value. Phenols, mainly the type of flavonoids from some medicinal plants are safe and bioactive and have antioxidants, antitumor, antibacterial and anti -inflammatory properties.

Table: 1.1 Phytochemical analysis of *Moringa pterygosperma* flower extracts

phytochemical constituents	floral extract *M.pterygosperma*	
	ethanolic	aqueous
Alkaloids	+	+
Flavonoids	+	+
Phenol	+	-
Saponins	-	-
Steroids	+	+
Tannins	-	-
Terpenoids	+	+
Glycoside	-	-

(+) presence; (-) absence

International Journal of Universal Science and Technology
ISSN: ISSN: 2454-7263 Copyright © Universal Print
Volume No. 03, Issue No. 05, Page No. 242-248 Published: Jan. 2018
Web: www.universalprint.org , Email: ijup@universalprint.org
Title Key: Evaluation of Phytochemical Screening …

Table 1.2: Free radical scavenging capacity of ethanol extract of *M.pterygosperma* flowers

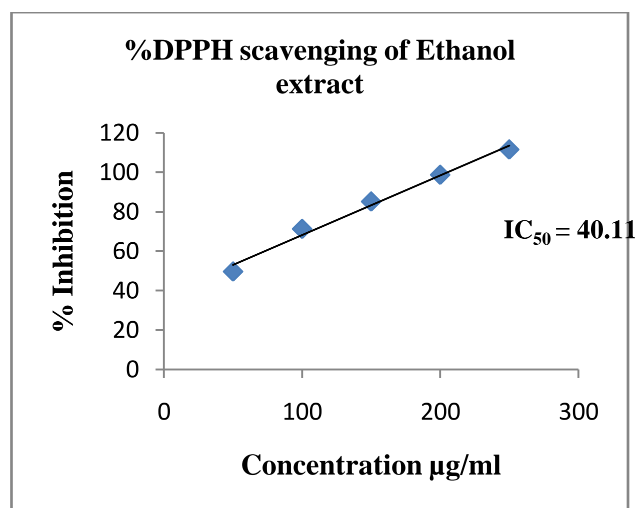
Concentration (µg/ml)	DPPH Scavenging %	
	Ethanol Extract	Ascorbic Acid
50	49.62±0.75	91.53±0.81
100	71.24±0.61	-
150	85.13±0.49	-
200	98.68±0.94	-
250	111.52±0.37	-
IC_{50}	40.11	-

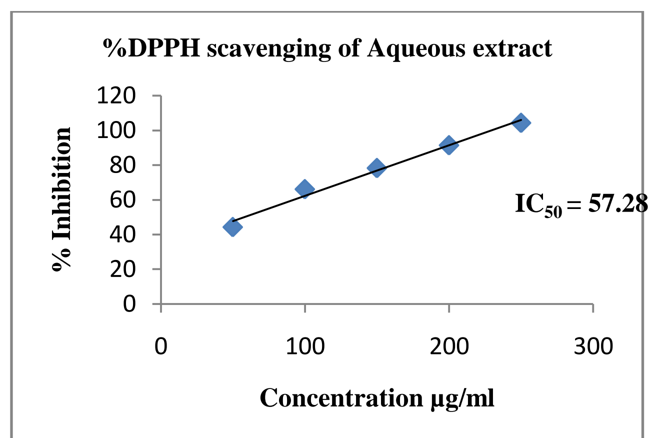
Values are mean ± SEM of three determinations

Table 1.3: Free radical scavenging capacity of aqueous extract of *M. pterygosperma* flowers

Concentration (µg/ml)	DPPH Scavenging %	
	Aqueous Extract	Ascorbic Acid
50	44.36±0.25	91.53±0.81
100	66.18±0.59	-
150	78.35±0.72	-
200	91.47±0.34	-
250	104.37±0.64	-
IC_{50}	57.28	-

Values are mean ± SEM of three determinations





4. Conclusion

The present study indicated that the extract from flowers of *Moringa pterygosperma* contain some medicinal active components. The ethanolic and aqueous extract of *Moringa pterygosperma* showed antioxidant activity by inhibiting DPPH and hydroxyl radical and total phenol content. The preliminary phytochemical investigation indicates the presence of phenol and flavonoids in the plant, and other bioactive constituent flavonoids type components which plays a major role in controlling antioxidants.

The result of this study show that the ethanolic and aqueous extract of *Moringa pterygosperma* can be used as easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. However, the components responsible for the antioxidant activity of ethanolic and aqueous extracts of *Moringa pterygosperma* are currently unclear. Therefore, further works have been performed on the isolation and identification of the antioxidant compounds present in extracts of plants.

5. Acknowledgement

The author are thank full to the principal Dr. Amit Roy and Dr. Ram Kumar Sahu, Department of pharmacy, Columbia Institute of Pharmacy , Raipur (C.G.) India for providing a laborotory for the experimenal work.

6. References

1. Farooq A. Sajid L., Muhammad, A. and Anwarul Hassan , G.; *Moringa Olifera* : A Food Plant with multiple medicinal uses , *Phytotherapy Research*, 21: 17-25 (2007).
2. Mughal, M.H., Ali. G. Srivasta P.S. and Iqbal M.; Improvement of Drumstick (*M. pterygosperma* Gaertn)" A unique source of food and medicinal through tissue culture. Harmdad Med. 42 : 37-42 (1999)
3. Fahey J.W., *Moringa Oleifera* : A review of the medicinal evidence for its Nutritional , Therapeutic and Prophylatic properties , *Trees life Journal* ,1 (2005)
4. Kawo, A.H. ;Water purification potential and In –vitro toxicity evaluation of the aqueous and petroleum ether extract of *Calotropis procera* and *Moringa Oleifera* Lam seed powder, Ph.D. Thesis, Microbiology unit, Department of Biological Science, Bayero University, Kano, 184 pp (2007).
5. Caceres A., Saravia A. , Rizzo S., Zabala L., Leon E.D. and Nave F.; Pharmacological properties of *Moringa oleifera 2:* Screening for antispasmodic, anti – Inflammatory and diuretic activity, *J.*Ethanopharmacol . 36: pp 233-237 (1992).
6. 6. Nickon F., Saud Z.A. , Rehman M.H. and Haque M.E.; In vitro antimicrobial activity of the compound isolated from chloroform extract of *Moringa oleifera* Lam., *Pak J. Biol. Sci.*, 22: 1888 – 1890 (2003).
7. Wiwit D.F., Taslim E. , Kuniyoshi S. and Sri Fatmawati ; Antioxidents activity of *Moringa* extract , *Indones J. Chem.* , 16 (3) : 297-301 (2016).
8. Larson R.; The antioxidant of Higher Plants, *Phytochemistry* , 27: 967-978(1988).
9. Sofawora E.A., ; Medicinal and Traditional medicine in Africa, *John Wiley and Sons*, London, 249 pp (1984).

10. Trease G.E. and Evans W.C. ; Pharmacognosy , 15th Edition , Saunders, London pp 53-336 (2002).
11. Harborne, J.B.,; *A Guide to Modern Techniques of plant analysis*, 3rd edn., London New York; Champan and Hall Phytochemical Method. (1998).
12. Braca A., De Tommasi N. , Di Bari L., Pizza C. Politi and Morelli I. ;Antioxidant principles from Bachinia Terapotensis , *J. Nat. Prod.* , 64 : pp 892-895 (2001)
13. Bargah R.K. and Kushwaha P.K.; Extractions , Phytochemical Screening and In- vitro Antioxidant activity of *Cassia fistula* Extracts , *International Journal of Research in Pharmacy and Chemistry,* 7(4) : 518-524 (2017)