Original Research Paper

Volume - 12 | Issue - 03 | March - 2022 | PRINT ISSN No. 2249 - 555X | DOI : 10.36106/ijar

Biotechnology

IN VITRO ANTIOXIDANT ACTIVITY OF *RAUVOLFIA MEDIA* BARKS AND LEAVES EXTRACTS

Razafison Lucrece Mamy Carlena Phd Student, Department Of Biotechnology , Faculty Of Applied Science, Parul University

ABSTRACT *Rauvolfia media* is a plant endemic to Madagascar, traditionally used against ulcers, diabetes and viral infections. No research has been scientifically conducted to prove the antioxidant activity of the plant. The current purpose of this study was to evaluate the antioxidant activity of *R. media* barks and leaves extracts with different solvents (acetone, acetic acid, methanol and water) by phosphomolybdate assay (total antioxidant capacity) and DPPH free radical scavenging. The results stipulate that the higher total oxidant capacity is found is *R. media* barks extracts which are acetic acid fraction with 0.332±0.01mg/g EAC, followed by the methanolic fraction with 0.816±0.01mg/g EAC ($p<0.001$). The DPPH data showed that the free radical scavenging activity increase with concentration, LMetOH fraction (20.711±0.01) had the greater free radical scavenging followed by LAcAc extract (28.375 ±1.2) with $p < 0.001$. The antioxidant activity can be attributed by the presence of certain phytochemical compounds in the plant extracts. According to these results, *Rauvolfia media* meets the criteria to be a natural antioxidant.

KEYWORDS : *Rauvolfia media,* antioxidant, total antioxidant capacity, radical scavenging

INTRODUCTION

In a normal human body, each body cell is attacked by an estimated 10, 000 to 20, 000 free radicals per day [1] For a free radical to neutralise itself, it seeks out and steal electrons from other molecules to gain stability [2], allowing this molecule to become a free radical itself [3] that is less harmful [4].

There are three sources of free radicals namely internal, external and physiological sources [5]. Internally generated sources of free radicals [4] include inflammation, xanthine oxide, phagocytosis, arachidonate pathways and ischemia/ reperfusions. Medicinal plants are qualified of having a good antioxidant activity by inhibiting free radicals and oxidation process which break free radical chain reactions. The chemicals present in the medicinal plant are compared with the most know antioxidant compounds as ascorbic acid and the poplyphenolic compounds [4].

The antioxidant activity of a substance is based on its ability to donate or accept hydrogen atom from the free radical. When free radicals are trapped, this terminates the chain reaction where by the reaction with reactive oxygen species is prevented thus maintaining the free radical in its redox state which leads to its inability to reduce molecular oxygen [6].

Literature reviews confirm that flavonoids, triterpenoids, steroids, steroidal glycosides, and alkaloids have been reported which shows antioxidant activity[7]. Flavonoids have been reported to be connected with antioxidative action in biological systems, performing as scavengers of singlet oxygen and free radicals [8].

Rauvolfia media belongs to genus *Rauwolfia* and family Apocyanaceae, that consists of around one thousand species [9], which most of them presents the antioxidant potential. The main goal of this study is to investigate the antioxidant activity of the plant parts extracts to prove its traditional uses.

MATERIALS AND METHODS

• Phosphomolybdate assay

The total antioxidant capacity of the fractions was determined by phosphomolybdate method using ascorbic acid as a standard [10]. An aliquot of 0.1ml of samples solution at different concentrations (100, 200, 300, 400 and 500mg/ml) were mixed with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes were them capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695nm against a blank. The blank contained 1ml of the reagent solution and the appropriate volume of the solvent and incubated under the same conditions. The experiments were conducted in triplicates and values are expressed as mg/g equivalent of ascorbic acid.

The antioxidant capacity was estimated using following formula:

Total antioxidant capacity (%)= [(A1-A0)/(A1)]x 100

With A0 represent the absorbance of extracts

A1 is the absorbance of control

• DPPH

The free radical scavenging activity of the extracts and ascorbic acid as positive control was measured. The method is based on hydrogen donating or radical-scavenging ability using the stable radical DPPH [11]. where a decolorization of DPPH from purple to yellow, indicate the scavenging effect of the extracts . 3mL of *Rauvolfia media* extracts and standard at various concentrations (200, 400, 600, 800, 1000 and 1200µg/ml) were added to 4 ml of freshly prepared DPPH solution (0.004%) in methanol. The reaction was incubated for 30min and the absorbance was measured at 517nm using a UV - visible spectrophotometer. All experiments were taken in triplicate. The percentage inhibition of DPPH free radical scavenging activity was calculated using the following equation:

Scavenging effect (%) = [(A1-A0)/A1] x 100

With A0 : absorbance of extracts

A1 : absorbance of control

EC50 value (µg/ml) for 50 % extract of the plant and DPPH concentration

• STATISTICAL ANALYSIS

Data are expressed as mean ± standard deviation. The EC50 values of different extracts were statistically analyzed on R studio software using One- way ANOVA test followed by Tukey's HSD post hoc test ($p<0.05$), which is considered as significant . .

RESULTS

The total antioxidant capacity were expressed as mg/g equivalent of ascorbic acid using a standard curve with an equation:

$y=0.0825x+0.5114$ ($R^2 = 0.9997$)

Table 1: Total Antioxidant Capacity Of *R. Media* Extracts

	BARKS	LEAVES
Ac	4.279 ±0.002	1.570 ±0.01
MetOH	0.816 ±0.001	3.424 ±0.003
AcAc	0.332 ±0	4.715 ±0.002
H2O	1.582 ±0.002	4.600 ±0.005

The assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically [12]. Table 1 shows the total antioxidant capacity of *Rauvolfia media* leaves and barks extracts. The BAcAc extract (0.332 ±0.01) showed high antioxidant capacity followed by the fraction BMetOH (0.816 ±0.001). However, the standard antioxidant activity was more effective than the *R. media* parts extracts (0.138 ±0.15).

DPPH radical scavenging assay is based on the reduction of DPPH in methanol solution in the presence of a hydrogen-donating antioxidant,

by a color change from purple to yellow, which is measured at 517 nm [13]. Table 2 demonstrates the scavenging effect of *R. media* barks and leaves extracts and the effect increase with the concentration. From the EC50 results (Figure 1), the DPPH radical scavenging activity of the LMetOH fraction (20.711 ±0.01) and LAcAc extract (28.375 ±1.2) were found to be significantly higher ($p < 0.001$). The EC50 value of BAc extract (34.469 ±0.24), BMetOH (35.212 ±0.5), BAcAc (39.575 ±0.01), LAc (45.125 ±0.47) and LH_2O fractions (54.935 ±0.1) was close as the standard, ascorbic acid (48.826 ±0.4). The plant extracts filled the criteria of an antioxidants.

Table 2: Dpph Scavenging Effect Of R. *Media* Extracts

C (µg/ml)	20	40	60	80	100	120
BARKS						
Ac	41%	55%	63%	73%	81%	92%
AcAc	41%	50%	59%	67%	75%	80%
MetOH	44%	52%	59%	63%	71%	77%
H_2O	24%	31%	42%	49%	57%	68%
LEAVES						
Ac	45%	48%	52%	58%	63%	68%
AcAc	46%	54%	65%	73%	81%	88%
MetOH	49%	58%	64%	73%	80%	86%
H_2O	30%	39%	51%	67%	80%	89%
ASCORBIC ACID						
	80%	87%	91%	94%	95%	98%

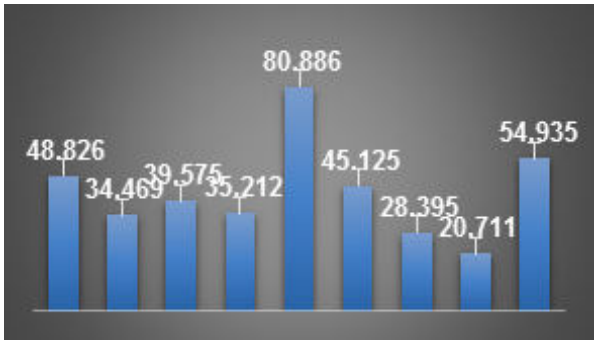

Figure 2: Ec50 Of Dpph Radical Scavenging

DISCUSSION

The antioxidant potential of the extracts was measured using the EC50 value which is 50% of the amount of extract needed to inhibit free radicals. Lower EC50 value indicates greater antioxidant activity [14].

The antioxidant potential of the parts of *R. media* extracts was estimated from their ability to reduce the reduction of Mo (VI) to Mo (V) by the antioxidant-enriched fractions and subsequent formation of a green phosphate/Mo (V) complex at acidic pH [15]. All the extracts showed a good total antioxidant activity that increased with increasing concentration.

The DPPH antioxidant activity of barks and leaves extracts of *Rauvolfia media* increased with an increase in concentration. Accordingly, in the present study maximum DPPH scavenging activity of *R. media* leaves and barks showed that all extracts had the same effect as ascorbic acid except the aqueous extract of barks and leaves and the acetonic leaves extract. The ability of an extract to act as free radical scavenger is due to its ability to neutralise free radicals to non-radical molecules, by their ability to donate hydrogen, accept free radicals, and interrupt chain oxidation reaction or chelate metals [16], thus terminating the chain of lipid peroxidation during oxidative stress [17].

Some articles reported that extracts containing some phytochemicals such as tannins, flavonoids, steroids, phenols and resins possessed an antioxidant activty [18].

CONCLUSION

Rauvolfia media antioxidant activity was shown by the results obtained from the phosphomolybdate assay and DPPH free radical scavenging assay. More investigation needs to be done for further study about which molecule is responsible for the activity. After the investigations, the medicinal plants can be used as a natural antioxidant.

REFERENCES:

[1] Marian Valko, Dieter Leibfritz, Jan Moncol, Mark T D Cronin, Milan Mazur, Joshua Telser. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol. 2006; 39(1), 44-84.
[2] Defeng Wu, Arthur I Cederbaum. Alcohol, oxidative stress, and free radical damage. Alcohol Res Health. 2003;27(4):277-84.
[3] Krishnamurthy, P., & Wadhwani, A. 2012. Antioxidant Enzymes and Human Health. Antioxidant Enzyme.
[4] Lobo, V., Patil, A., Phatak, A., & Chandra, N. . Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews, 2010; 4(8), 118.
[5] Kumar MS, Balachandran S, Chaudhury S. Influence of Incubation Temperatures on Total Phenolic, Flavonoids Content and Free Radical Scavenging Activity of Callus from Heliotropiumindicum L. Asian J. Pharm. Res. 2012; 2(4), 148-152.
[6] Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: a review with recent updates. Interdisciplinary Toxicology, 5(2).
[7] Sahu NP, Koike K, Jia Z, Nikaido T: Triterpenoid saponins from Mimusops elengi. Phytochemistry 1997; 44 (6), 1145-9.
[8] Jorgensen LV, Madsen HL, Thomsen MK, Dragsted LO and Skibsted LH: Regulation of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy. Free Radical Research.1999; 30:207-220.
[9] Bhattacharjee SK: The Handbook of Medicinal Plants, Pointer Publishers, India, 2004.
[10] Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999;269:337–41.
[11] Susanti D, Sirat HM, Ahmad F, Ali RM, Aimi N: Antioxidant and cytotoxic flavonoids from the flowers of Melastoma malabathricum L. Food Chem. 2007, 103: 710-716. 10.1016/j.foodchem.2006.09.011.
[12] Blois M.S. Antioxidant determinations by the use of a stable free radical. Nature. 1958; 181:1199–1250.
[13] Umamaheswari M., Chatterjee T.K. In vitro antioxidant activities of the fractions of Coccinnia grandis L. leaf extract. Afr J Trad Complement Altern Med. 2007 Oct;5(1):61–73.
[14] Raghavendra, M., Reddy, A.M., Yadav, P.R., Raju, A.S. and Kumar, L.S., 2013.Comparative studies on the in vitro antioxidant properties of methanolic leafy extracts from six edible leafy vegetables of India. Asian Journal of Pharmaceutical and Clinical Research, 6, pp.96-99.
[15] Khan RA, Khan MR, Sahreen S. Assessment of flavonoids contents and in vitro antioxidant activity of Launaeae procumbens. Chem Central J. 2012;6:43.
[16] Viuda-Martos, M., Ruiz-Navajas, Y., Fernández-López, J. and Pérez-Álvarez, J.A., 2010. Effect of adding citrus fibre washing water and rosemary essential oil on the quality characteristics of a bologna sausage. LWT-Food science and Technology, 43, pp.958- 963.
[17] Dutta, A.K., Gope, P.S., Makhnoon, S., Rahman, M.S., Siddiquee, M.A. and Kabir, Y., 2012. Effect of Solvent extraction on Phenolic content, Antioxidant and-Amylase Inhibition activities of Swertia chirata. International Journal of Drug Development and Research, 4, pp.317-325.
[18] Assimopoulou, A.N., Zlatanos, S.N. and Papageorgiou, V.P., 2005. Antioxidant activity of natural resins and bioactive triterpenes in oil substrates. Food chemistry, 92, pp.721-727.