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ANTIOXIDANT PROPERTIES OF THE METHANOL EXTRACTS OF LEAF, STEM, FRUIT AND SEED OF NIGERIAN *HARUNGANA MADAGASCARIENSIS*, AN HYPERICACEAE (GUTIFERAE)

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ABSTRACT

The leaf, stem, fruit and seed of Nigerian *Harungana madagascariensis* (an Hypericaceae), were exhaustively extracted with methanol to obtain their methanol extracts designated as MHmL, MHmSt, MHmF and MHmSd respectively. The extracts were subjected to assessment of their antioxidant activity using 2,2-diphenyl-1-picryl hydrazyl (DPPH), while Vitamin C, Vitamin E and Butylated hydroxyl anisole (BHA) were used as standards. The four methanol extracts displayed higher antioxidant activity than Vit E., while the stem methanol extract (MHmSt), showed the highest antioxidant activity (IC50 = 0.0250 (mg/mL) among the four. These results provide substantial basis for the ethno-medicinal applications of the plant. The long-term aim of our present research seeks at discovering new natural antioxidant compounds from this highly ethno-medicinally utilized plant.

KEYWORDS: *Harungana madagascariensis*, Hypericaceae, Antioxidant, DPPH.

INTRODUCTION

Harungana madagascariensis Lam. ex Poiret is a 'Hypericaceae', earlier classed as 'Guttiferae'. It is a tropical much-branched shrub to small tree, which grows up to 12 m tall, commonly known as 'Dragon's blood tree'. The plant is very useful in Africa, native to Madagascar, Mauritius, growing on margins of wet forests. *H. madagascariensis* is made of bright orange bark exudates and distinctive broad egg-shaped opposite leaves which are 10 to 20 cm long and 6 to 10 cm wide. It has fragrant flowers, which are very small, whitish with black glands; its orange to brown fleshy fruits are small, about 2 to 3 mm and have 2 to 4 seeds in them. The wood colored orange-red to yellow is very attractive.[1,2,3,4]

H. madagascariensis has the following adaptive features for its survival: it forms dense thickets from root suckers for exclusion of all other species around, as well as invades cyclone-damaged and gaps in rainforest. It can also re-grow after disturbance and is capable of withstanding poor drainage on alluvium. The plant is a vigorous colonizer, hence named '*Harungana madagascariensis*' and is also referred to as a pest plant.[2,3,4,5a,5b]

H. madagascariensis has many social and medicinal applications: the light wood is utilized in construction of huts, store-houses, thwarts and seats in canoes, yam-sticks and hockey-sticks; its wood serves as fuel in local metallurgy; the gummy delicate dye is utilized for dying velvet, also a good stain for wood and sealing-wax on newly fired pots.[2,3,5a,5b]

Harungana madagascariensis is very useful therapeutically. It has been reported to possess antimalarial, antibacterial, antifungal, and antiviral activities.[6,7,8] In agriculture, *H. madagascariensis* is utilized in the prevention of poultry illnesses; ethno-medicinally it is applied in cures for leprosy, jaundice, ulcers, asthma, stomachache, cough, dysentery and piles. Its gum is styptic and hemostatic, for arresting blood flow, it is even applied to cuts and for dressing wounds. *H. madagascariensis* serves as remedy for reproductive disorders like acting as placental embolic and emmenagogue. It relieves painful menstruation and menstrual problems, miscarriage, sterility and hematuria.[9-13] It combats hemorrhage, diarrhea, gonorrhea, sore throat and fevers.[9-15] The leaf and roots of *H. madagascariensis* are considered febrifugal and anti-malarial. Leaf-sap is prepared as a remedy for amenorrhea and heart-troubles, as laxative for stomachic and intestinal troubles. Ointment made from *H.*

madagascariensis fruit in animal fat is used on inflamed ganglia. *H. madagascariensis* has been shown to have hypoglycemic effects, lowering blood glucose levels in diabetes mellitus. It is also known to have anti-inflammation, antioxidant, anti-hepatotoxicity and antimicrobial activities.[16-21]

There are reports on screening phytochemicals in *Harungana madagascariensis*.[24,25] The leaf and stem essential oils are highly terpenoidal, dominated by sesquiterpenes.[26] The following compounds have been isolated from different parts of *Harungana madagascariensis*: quercetin, β-sitosterol, madagascarin, madagascin, madagascin anthrone, vismiaquinone, betulinic acid, friedelin, euxanthone, chrysophenol, physcion, harunganin, harunganin anthrone, anthranoids, harongin anthrone, harunganol A, harunganol B, kenganthranol A,B and C, 1,7-dihydroxyxanthone, aloe-emodin-ω-acetate, astilbin, and prenylated 1,4-anthraquinone (kengaquinone).[6,8,27-34]

Antioxidants are widely useful in providing protection against oxidative degradation. The mechanism is attributed to its strong hydrogen donating ability, a metal chelating ability, and their effectiveness as good scavengers of hydrogen peroxide, superoxide, and free radicals. In addition, phenolic compounds appear to be the components responsible for the antioxidant activities, which supports anti-aging and freshness in animals. Some earlier reports agree with this.[25,35,36] Therefore it is important to have assessments of bioactivities including antioxidant evaluations on extracts along with isolated and identified compounds to be able to assess and explain better the mechanisms of antioxidant activities, as well as other bioactivities. Our present study and further research studies are aimed at this. We report the antioxidant activities of four extracts that are important in the ethno-medicinally utilized Nigerian *Harungana madagascanensis*, which has not been earlier reported.

MATERIALS AND METHODS/EXPERIMENTAL

Plant collection and identification

Fresh samples of *Harungana madagascariensis* were collected from a forest reserved area in Ibadan, Nigeria, for extractions. The plant was authenticated at the Herbarium, Department of Botany, University of Ibadan, where voucher samples have been deposited, with voucher number UIH – 22455. The plant samples were separated into different parts, and then air dried for two weeks, and pulverized to powder.

Extraction of plant

Each of the pulverized plant parts, were exhaustively extracted with methanol by repeated soaking for ten days. Each extract was concentrated to dryness using a rotatory evaporator (RE 100 CO S35, Bibby Scientific Ltd). The extracts were stored in glass vials prior to the antioxidant analyses.

Antioxidant activity

The DPPH (2, 2- diphenyl-1-picrylhyrazyl) free radical scavenging activity measurements were done according to standard methods.[37] 3.94 mg of DPPH was dissolved in 100 mL methanol to obtain a 0.1 mM solution. A stock solution of 0.50 mg/mL of each test plant sample was made and from it was obtained other concentrations (0.25, 0.13, 0.06 and 0.03 mg/mL) by serial dilutions.

2 mL of each test plant solution was added to an equal volume of the DPPH solution in a screw cap test tube. The test tubes were closed, well shaken and placed in a dark cupboard for 30 minutes. Similarly, a blank reaction mixture consisting of 2 mL of methanol without the plant samples and an equal volume of DPPH solution was prepared. After 30 minutes, the absorbance of each mixture at 517 nm was measured on a Shimadzu UV-2101 PC, UV-VIS Scanning Spectrophotometer.

Free radical scavenging activities were calculated as a decrease in the absorbance of DPPH using the following equation:

% Inhibition = [(AB-AS)/AB] x 100

Where AB = Absorbance of blank and AS = Absorbance of sample

RESULTS AND DISCUSSION

In an earlier study, the four methanol extracts were screened and evaluated for their classes of phytochemicals. Phytochemical screening of the four extracts using standard methods showed the presence of high amount of tannins, saponins, terpenoids, alkaloids, flavonoids, reducing sugars, resins, cardiac glycosides and phenols.[24]

In this study, total antioxidant activities were determined by the DPPH assay. The % inhibitions for the extracts ranged from 67.67 to 99.40 %, while that of the standards were from 63.36 to 97.09 %. Antioxidant activities were found to be generally concentration dependent. All four *Hm* extracts displayed higher DPPH inhibition than Vit E which is a known standard for antioxidant assessments (IC50 = 0.0377 mg/mL). The DPPH inhibition of the *Hm* extracts were comparable to that of vitamin C and BHA at higher concentrations.

The stem methanol extract (MHmSt) gave the highest antioxidant activity (IC50 = 0.0250 mg/mL), followed by the fruit (MHmF IC50 = 0.0262 mg/mL); then the seed (MHmSd IC50 = 0.0333 mg/mL) and lastly the leaf (MHmL IC50 = 0.0356 mg/mL).

The percentage inhibitions are presented in the bar chart and graphical representations below:

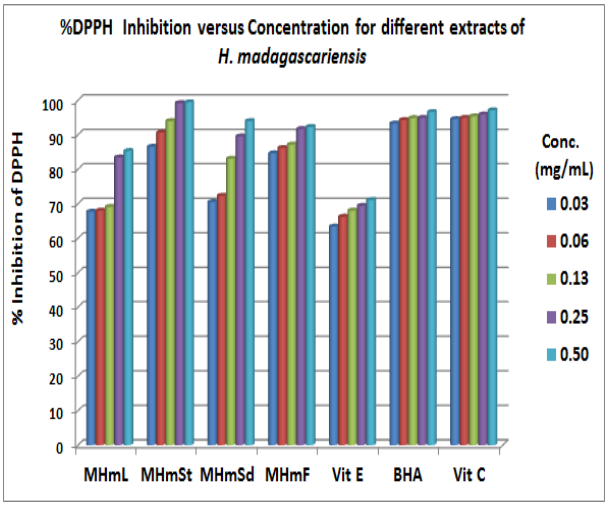

Fig. 1: Bar Chart Representation.

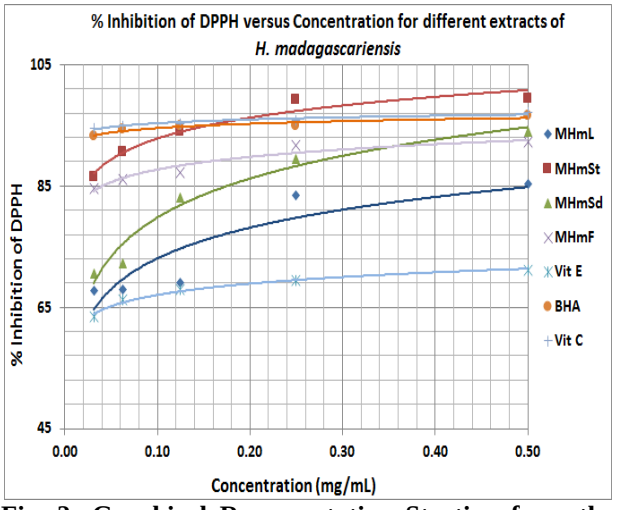

Fig. 2: Graphical Representation Starting from the Concentrations Used.

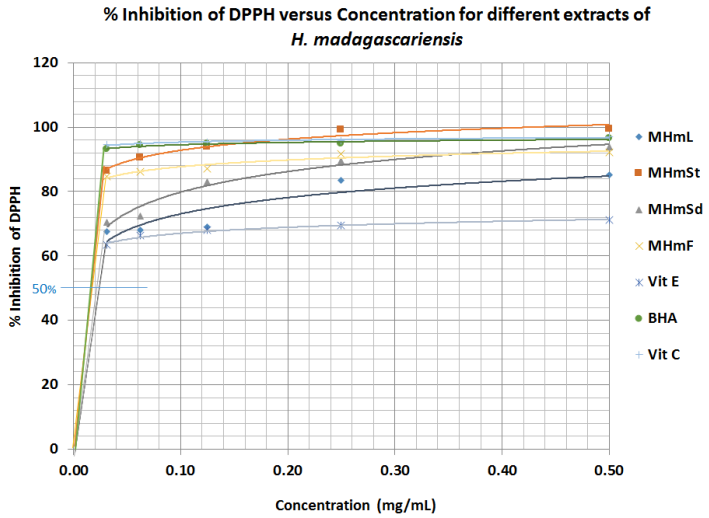

Fig. 3: Graphical Representation Starting from Origin (Zero).

Table 1: IC_{50} (mg/mL) Values.

	MHmL	MHmSt	MHmSd	MHmF	Vit E	BHA	Vit C
IC_{50} (mg/mL)	0.0356 ± 0.0100	0.0250 ± 0.0093	0.0333 ± 0.0096	0.0262 ± 0.0097	0.0377 ± 0.0087	0.0230 ± 0.0101	0.0226 ± 0.0102

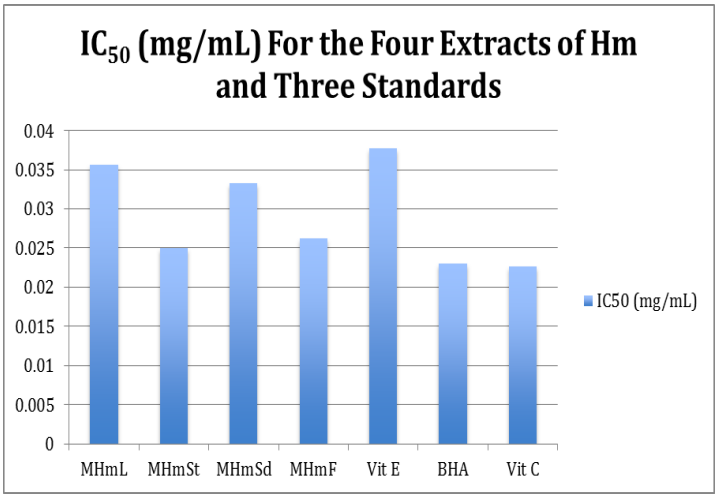

Fig. 4: Bar Chart of IC_{50} (mg/mL) Values.

CONCLUSION

All extracts displayed DPPH inhibition comparable to the standards vitamin C and BHA. All the extracts of *H. madagascariensis* displayed higher DPPH inhibition than Vitamin E. The extract MHmSt showed the highest DPPH inhibition of all the samples tested (99.46%).

The results show that the extracts of *H. madagacariensis* have promising potential to be formulated for use as radical scavenging antioxidants.

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REFERENCES

1. Queensland Government. Harungana is a declared Class 3 pest plant under Queensland legislation. Primary industries and fisheries. Weeds, pest animals and ants. DEEDI Home, 2010.
2. Burkill HM. The useful plants of west tropical Africa. *Harungana madagascariensis* Lam. ex Poir. [family GUTTIFERAE]. Guttiferae, 1985; 2.
3. Royal Botanic Gardens, Kew. Useful plants of west tropical Africa. *Harungana madagascariensis* Lam. ex Poir. [family GUTTIFERAE]. Guttiferae, 1990.
4. Orwa CA, Mutua A, Kindt R, Jamnadass R, Anthony S. AgroforestreeDatabase:a tree reference and selection guide version 4.0. Agroforestry Database, 2009. 4.0. (http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp).
5. Neuwinger HD. (a) In African Ethnobotany: Poisons and Drugs- *H. madagascariensis*, Chapman and Hall, London.1996. (b) African Traditional Medicine, a Dictionary of Plants Uses and Applications. 1st Edn. Medpharm Scientific Publishers, Stuttgart. 2000. ISBN: 978-3887630867.
6. Lenta BN, Ngouela S, Bovom FF, Tantangmo F. Antiplasmodial activity of some constituents of the root bark of *H. madagascariensis* Lam. (Hypericaceae). Chem. Pharm. Bull., 2007; 55(3): 464-467.
7. Moulari B, Lboutounne H, Chaumont JP, Guillaume Y, Millet J, Pellequer Y. Potentiation of the bactericidal activity of *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae) leaf extract against oral bacteria using poly (D, L-lactide-co-glycolide) nanoparticles: in vitro study. Acta Odontol Scand., 2006; 64: 53-158.
8. Moulari B, Pellequer Y, Lboutounne H, Girard C, Chaumont JP, Millet J, Muyard F. (2006). Isolation and in vitro antibacterial activity of astilbin, the bioactive flavanone from the leaves of *Harungana madagascariensis* Lam. ex Poir. (Hypericaceae). Journal of Ethnopharmacology, 2006; 106: 272-278.
9. Bouquet A, Debray M. Trav. Doc. Orstom., 1974; 32: 1-11.
10. Vasileva B. Plantes Medicinales de Guinee, Conakry, Republique de Guinee. Merck's Explorer Magazine, 1969. <magazine.merckgroup.com/Harunganamadagascariensis>.
11. Iwu MM. In Handbook of African Medicinal Plants, CRC Press, Boca Raton, Fla., 1993.
12. Hakizamungu E, Puyvelde LV, Wery M. Screening of Rwandese medicinal for antitrichomonas activity. J. Ethnopharmacol., 1992; 36: 143-146.
13. Madubunyi II, Obi SKC, Nwebube NI, Chime AB. International Journal of Pharmacognosy, 1995; 33(2): 129-134.
14. Akah PA. Abortifacient activity of some Nigerian medicinal plants. Phytotherapy Res., 1994; 8(2): 106-108.
15. Kokwaro JO. Medicinal Plants of East Africa, 3rd Edition. In: Proceedings of 5 of African Crop Science Conference, Lagos, Nigeria. University of Nairobi Press., 2009.
16. MC Gessler. Screening Tanzanian medicinal plants for antimalarial activity. ActaTropica., 1994; 56(1): 65-77.
17. Gessler MC. Tanzanian medicinal plants used traditionally for the treatment of malaria. In vivo antimalarial and in vitro cytotoxicity activities. Phytotherapy Res., 1995; 9: 504-508.
18. Abbiw DK. In Useful Plants of Ghana, Intermediate Technology Publications and The Royal Botanic Gardens, Kew, London, 1990; S: 154-157.
19. Maikere-Faniyo R, Puyvelde LV, Mutwewingabo A, Habiyaremye F. Study of Rwandese medicinal plants used in the treatment of diarrhea. J. Ethnopharmacol., 1989; 26(2): 101-109. [PubMed].
20. SureChem. Method of reducing blood glucose by administering Harunganin. 1998; Patent Number: 5837255. Issue Date: 1998-11-17. (1998).
21. Adeneye AA, Olagunju JA, Elias SO, Olatunbosun DO, Mustafa AO, Adeshile OI, Ashaolu AO, Laoye TA, Bamigboye AO, Adeoye AO. International Journal of Applied Research in Natural Products, 2008; 1(3): 29-42. http://www.healthy-synergies.com.
22. Iwalewa EO, Naidoo V, Bagla V, Eloff JN. African Journal of Pharmacy and Pharmacology, 2009; 3(1): 014- 021. Available online http://www.academicjournals.org/ajpp. ISSN 1996-0816 © 2009 Academic Journals.
23. Iwalewa EO, Adewale IO, Taiwo BJ, Arogundade T, Osinowo A, Daniyan OM, Adetogun GE. Journal

of Complementary and Integrative Medicine, 2008; 5(1): 1553-3840. DOI: 10.2202/1553-3840.1088.
24. Moronkola DO, Olaoluwa OO, Oladosu IA, Aboaba SA, Aiyelaagbe OO. Phytochemical and Antimicrobial Activities of Extracts from Six Medicinal Plants Utilized as Antimalarials in Ethno-Medicine. The Pharmaceutical and Chemical Journal, 2018; 5(2): 52-61. ISSN: 2349-7092 CODEN (USA): PCJHBA. www.tpcj.org.
25. Biapa PCN, Agbor GA, Oben JE, Ngogang JY. Phytochemical studies and antioxidant properties of four medicinal plants used in Cameroun. Afr. J. Trad. CAM., 2007; 4(4): 495-500.
26. Moronkola DO, Yeboah SO, Majinda RRT, Sichilongo K. Compositions of *Harungana madagascariensis* Lam. ex Poiret leaf and stem essential oils. Journal of Chemical and Pharmaceutical Research, 2015; 7(5): 959-964.
27. Kouam SF, Ngadjui BT, Krohn K, Choudhary MI. Thermal rearrangement of harunganin and allylations of some compounds in *Harungana madagascariensis*. ARKIVOC, 2007; ix: 64-70. ISSN 1424-6376 Page 64-70 ©ARKAT USA, Inc. Issue in honour of Prof Berhanu Abegaz.
28. SF Kouam; BT Ngadjui; K Krohn; P Wafo; AAjaz; MI Choudhary. Prenylated anthronoid antioxidants from the stem bark of *Harungana madagascariensis*. Phytochemistry, 2005; 66(10): 1174-1179. Science Direct. Elselvier.
29. Stout GH. Harunganin: J. Am. Chem. Soc., 1962; 84: 2653-2654.
30. Eze FN, Airouyuwa OJ. Composition of *Harungana madagascariensis* Lam. Ex Poiret. British Journal of Pharmaceutical Research, 2014; 4(17): 2068-2074. ISSN: 2231-2919. Science domain international www.sciencedomain.org.
31. Afieroho OE, Izontimi SS, Okoroafor DO, Caleb B. *Harungana madagascariensis* Lam Ex Poir (Hypericaceae) Fruits Oil Extract and Acute Toxicity Evaluation. International Research Journal of Pharmacy, 2012; 3(11): 75-77. www.irjponline.com ISSN 2230 – 8407.
32. Tih AE, Ghogomu RT, Sondengam BL, Caux C, Bodo B. Minor constituents of *Harungana madagascariensis* stem bark. Biochemical Systematics and Ecology, Science Direct. Elselvier, 2006; 34: 267-269.
33. Kouam SF, Khan SN, Krohn K, Ngadjui BT, Kapche DGWF, Yapna DB, Zareem S, Moustafa AMY, Choudhary MI. α-Glucosidase inhibitory anthranols, kenganthranols A-C, from the stem bark of *Harungana madagascariensis*. J. Nat. Prod., 2006; 69(2): 229-233. DOI: 10.1021/np050407n.
34. Iinuma M, Tosa H, Ito T, Tanaka T, Aqil M. Two prenylated anthrones in *Harungana madagascariensis*. Phytochemistry, Pergamon, 1995; 40(1): 267-270.
35. Ipav SS, Ibok NU, Dasofunjo K. Phytochemical screening and free radical scavenging activities of the fruit and leaves of *Kigelia Africana* (Bignoniaceae). ARPN Journal of Science and Technology, 2014; 4(2): 123-128.
36. Gulcin I. The antioxidant and radical scavenging activities of black pepper (*Piper nigrum*) seeds. International Journal of Food Science and Nutrition, Taylor and Francis, 2005; 56(7): 491-499.
37. Ajiboye CO, Moronkola DO, Adesomoju AA. DPPH free radical scavenging activities of leaf, stem bark, root, flower and fruit of *Blighia unijugata* Baker (Sapindaceae) extracts. Journal of Chemical, Biological and Physical Sciences, 2017; 7(4): 1190-1197.