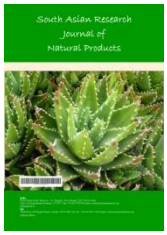

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Phytochemical Screening, Anti-microbial Activity and Chromatographic Studies of Parts of *Harungana madagascariensis*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study highlights the use of plants as an important component of the health care system in Nigeria. Parts of *Harungana madagascariences*, one of the medicinally important plants commonly found in Nigeria, was phytochemically and chromatographically evaluated in the present work for the identification of various phytochemical compounds found in it. The phytochemical tests showed the presence of alkaloids, tannins, steroids, cardiac glycosides, flavonoids and carbohydrates in extracts of *H. madagascariences*. Antibacterial activity of the plant extracts revealed that the ethyl acetate and methanol extracts showed some degree of inhibition of growth in *Staphylococcus aureus, Salmonella typhi, Escherichia coli and Shigella sonnei.* A total of twenty Seven (27) compounds were identified through spectrum matching with National Institute Standard and Technology (NIST) database in the methanol fraction of *H. madgascariensis* leaf extract by GC-MS analysis. Compounds identified included 2-(2-hydroxy-2-phenylethoxy) phenol, n-hexadecanoic acid, 4-hydroxy-N_2-(4,5,6,7-tetrahydro-5-hydroximinobenzofurazan-4-ylidino)-benzhydride, Nortricyclanol, acetylmonoglyceride, 3-methyl-4- (phenlthio) – 2-prop-2-enyl-2,5-dihydrothiophere 1,-1-dioxide, Z,Z-2,6-dimethyl-3,5,7-octatriene-2-ol, Glycerol, 3-deoxy-d-mannonic acid, 10,13-dioxo-tricyclo (6:3:3:0) tetradec-4-ene and 3,4-altrosan. The presence of these compounds in the leaf methanol extract (LME) justifies the use of *H. madagascariences* as a good source of therapeutic

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agents for bacterial and fungal infections. Also, it is rich in compounds which possess anti-oxidant, anti-spermatogenic, anti-biotic and neuroprotective activity. *H. madacascariences* is found to be a pharmacologically important plant, hence, further isolation of individual phytochemical constituents and subjecting them to the biological activity will give more pharmaceutically valuable results.

Keywords: GC-MS analysis; Harungana madagascariences; methanol fraction; anti-bacterial activity.

1. INTRODUCTION

Before the advent of orthodox medicine, most societies totally depended on traditional medicines for their health care needs. For most rural dwellers in Nigeria, even in the present age, the story has not changed. The people still depend, in most cases, on the traditional medicine practitioners who, in turn, rely on plants that have the therapeutic values.

These plants, which for centuries, have been in solitude and obscurity in the forest are becoming popular. The reason for this popularity is that they now have proven values in providing succor to the sick. As a result, scientists are turning to these tropical plants and traditional medicine. Another reason for the popularity of these medicinal plants is that the microorganisms responsible for illnesses are now becoming more resistant to a number of antimicrobial drugs. There is, therefore, the need to hunt for new ways of tackling illnesses and diseases such as cancer, liver problems, typhoid fever, diabetes, malaria, anaemia, HIV/AIDS, among others. One way of doing this is through phytochemistry in order to develop new drugs to complement the already existing ones on the pharmacist's shelf.

Harungana madagascariensis, commonly known as blood tree or orange milk tree is indigenous to Africa, particularly Central African Republic, Congo, Democratic Republic of Congo, Ethiopia, Kenya, Lesotho, Madagascar, Namibia, South Africa and Nigeria. However, the plant is now found in Australia [1].

Traditional medicine practitioners have claimed that *H. madagascariensis* is effective in the treatment of scabies, constipation and as anthelmintic (tape worms expellers). The leaves have been used as a remedy for hemorrhage, diarrhea, gonorrhea, sore throat, headache and fever. Resin from the flower stalks is believed to ease infant colic and to check infection after child birth decoction of the plant root and stem is also used as remedy for dysentery, bleeding, piles, trypanosomosis, typhoid, fever, cold, cough diabetes and jaundice [2].

Extracts from the roots of the plant have been used to hasten breast development in young women. Also, the roots and stem bark are boiled in water, and infusion drunk twice a day to interrupt menstrual flow and postpartum bleeding. The young leaves of the plant are used to arrest asthma [3].

Agbor [4] reported that the leaf of *H. madagascariensis* contains saponins, tannins, phenol, oils, steroids and flavonoids.

The effectiveness of *H. madagascariensis* against some pathogens has been confirmed by modern scientific studies. The antimicrobial effect of the plants leaf ethanolic, chloroform and petroleum ether extracts showed that the organic solvents extracts elicited inhibitory activities on *S. typhi* and *S. aureus* used; while *E. coli, K. pneumonea* and *P. aeruginosa* showed resistance to all the extracts [5]. The minimum inhibitory concentration (MIC) value of the extracts on the organisms ranged between 0.0625 - 0.125 mg/ml for *S. typhi* and 0.125 – 0.25 mg/ml for *S. aureus* [5].

The DPPH free radicals scavenging ability, antioxidant property and iron (II) chelating abilities of the bark of *H. Madagascariensis* has also been reported [6,7]. *H. Madagascariensis* has been shown to be used in human and ethnoveterinary medicine as an antiparasitic, anti-anaemic, spasmoltic and anti-bacterial in skin disease and wounds [8,9].

In platelet aggregation study, it has been deduced that the constituents in *H. madagascariensis* stem bark has a dual role of activities: At low Nitrogen (II) oxide (NO) concentration, the oxide can exhibit or induce platelet aggregation, but at high concentration Nitrogen (II) oxide being free radical oxygen species, can decrease platelet aggregation and in another dimension, it can therefore induce cell death and thus produce inflammation [10].

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation of Materials

The fresh leaves, bark and roots of the plant were harvested from their natural habitat in Jibam village of Pankshin, LGA of Plateau State. The plant materials were dried under shed for three weeks before being ground into powder. The powdered materials were stored in sterile polyethene bags until required for use.

2.2 Extraction of Plant Materials

Analar grades of n-hexane, ethyl acetate and methanol were used for the extraction.

500g samples of each plant materials were macerated in 250cm^3 of each of the solvents. The solvents used for extraction were n-hexane, ethyl acetate, methanol and water in that order. Each of the extraction mixtures was left for 24hrs in a tight fitted bottle. And the extracts were filtered under gravity. The process was repeated with the same plant materials several times until it was clear that the extraction was completed. Each plant material was then dried and the process was repeated with ethyl acetate, methanol and finally, water. The extracts were concentrated using sohxlet apparatus and the concentrated extracts were placed in a desiccator to further dry. The dried extracts were put into sample bottles for further analysis.

2.3 Preliminary Phytochemical Screening

The preliminary phytochemical screening was performed on the crude extracts using standard basic tests for phytochemicals [11,12]. The plant extracts were screened for the presence of alkaloids, saponins, tannins, steroids, cardiac glycosides, flavonoids, anthraquinones and carbohydrates.

2.4 Bioassay of Crude Extract

The test organism used for sensitivity test included four bacteria. They were *Staphylococcus aureus, Salmonella typhi, Escherichia coli and Shigella sonnei.* The choice of these pathogens was based on their implication in human diseases such as typhoid fever, enteric fever, pneumonia, urinary tract infections, dysentery, respiratory problems, wound infections and others. These ailments have been endemic especially in third world countries, like Nigeria.

The pathogens were used to test the activities of the plant “crude extracts”.

2.5 Chromatographic Separation of the Isolates from *Harungana madagascariensis* Crude Extract

Column chromatography was used to separate the pure samples (isolates) from the crude extracts following the recommended methods [13].

Phytochemical screening of the isolate further confirmed presence of alkaloids, saponins, tannins, steroids, cardiac glycosides, flavonoids, anthraquinones and carbohydrates. A total of 31 fractions were collected from the column. These fractions were pooled together into a group of six fractions according to their polarity. Table 3 summarizes the results.

2.6 Bioassay of Isolates from *H. madagascariensis*

The test organism used for the sensitivity test for the chromatography fractions includes *Staphylococcus aureus, Salmonella typhi, Escherichia coli* and *Shigella sonnei*. The result shown in Table 4 revealed that only *S. aureus* was sensitive to the fractions of the leaf methanol extract (LME).

3. RESULTS AND DISCUSSION

The result of the phytochemical screening shown in Table 1 revealed the presence of alkaloids, tannins, steroids, cardiac glycosides, flavonoids, saponins and carbohydrates. Anthraquinones and saponins were not detected in any of the crude extracts. Alkaloids were present in all the extracts except leaf hexane extract, leaf water extract, stem ethyl acetate extract, stem water extract, root water extract and whole root. Leaf methanol extract contained Alkaloid, Saponin, tannin, flavonoid carbohydrate, steroids and cardiac glycosides. This was in agreement with previously reported results which showed that the leaf of *H. madagascariensis* contained saponins, tannins, phenols, oils, steroids and flavonoids [4].

Table 1. Result of the preliminary phytochemical screening of the extract of *Harungana madagascariensis*

Extract	Alkaloids	Saponins	Tannin	Flavonoids	Carbohydrates	Steroids	Anthraquinones	Cardiac glycosides	Colour of extract
LHE	+	-	-	-	-	++	-	++	Dark green
LEE	-	-	-	-	-	+++	-	+++	Dark green
LME	++	-	+++	+++	+++	++	-	+++	Reddish
LWE	-	-	++	++	+	-	-	-	Reddish brown
SHE	+	-	-	-	+	+++	-	-	Milk
SEE	-	-	-	-	+	++	-	+++	Yellowish brown
SME	++	-	++	++	+++	-	-	++	Brown
SWE	-	-	+++	++	+	+	-	-	
RHE	+	-	-	-	-	+++	-	++	Light Yellowish
REE	+++	-	-	-	+	+	-	+++	Dark brown
RME	+++	-	++	++	++	-	-	++	
RWE	-	-	-	-	-	-	-	-	
Leaf	+++	-	++	+++	+++	+	-	+	
Whole stem	+++	-	++	+++	+++	+	-	+	
Whole root	-	-	+++	++	-		-	-	

Key: LHE- leaf hexane extract, LEE- leaf ethyl acetate extract, LME- leaf methanol extract, LWE- leaf water extract, SHE- stem hexane extract, SEE- stem ethyl acetate extract, SME- stem methanol extract, SWE- stem water extract, RHE-root hexane extract, REE- root ethyl acetate extract, RME- root methanol extract, RWE- root water extract

Table 2. Antibacterial Activity of the Extracts against some selected organisms at an approximate concentrations

	Diameter of zones of inhibition (mm)			
Extracts	**Ec**	**Sa**	**Sal**	**Shg**
Lee	12	14	12	20
See	15	17	20	18
Ree	7	7	0	0
Lhe	0	0	0	0
She	0	0	0	0
Rhe	0	0	0	0
Lwe	0	0	0	0
Swe	0	0	0	0
Rwe	0	0	0	0
Lme	22	28	14	18
Sme	24	26	22	30
Rme	22	14	12	20
Ciprofloxacin	32	27	28	31

Key: Sa= Staphylococcus aureus; Sal= Salmonella species; Ec= Escherichia coli; Shg= Shigella species

Table 3. Pooled column chromatography fractions of leaf methanol extract of H. madagascariensis

Fractions	**Solvent**
LMe_1 (1-13)	Hexane (100%)
LMe_2 (14-18)	Hexane: Chloroform (50:50)
LMe_3 (19-22)	Chloroform: methanol (90:10)
LMe_4 (23-27)	Chloroform: methanol (80:20)
LMe_5 (28-30)	Chloroform: methanol (50:50)
LMe_6 (31)	Methanol (100%)

Table 4. Result of the Bioassay with LME of *H. madagascariensis* fractions (pooled column chromatography fractions)

	Diameter of zone of inhibition						
Pathogen	**LME1**	**LME2**	**LME3**	**LME4**	**LME5**	**LME6**	**CPR**
Sa	8	8	22	20	0	28	27
St	-	-	-	-	-	-	28
Ec	-	-	-	-	-	-	32
Shig.	-	-	-	-	-	-	31

Key: CPR – Ciprofloxacin (control)

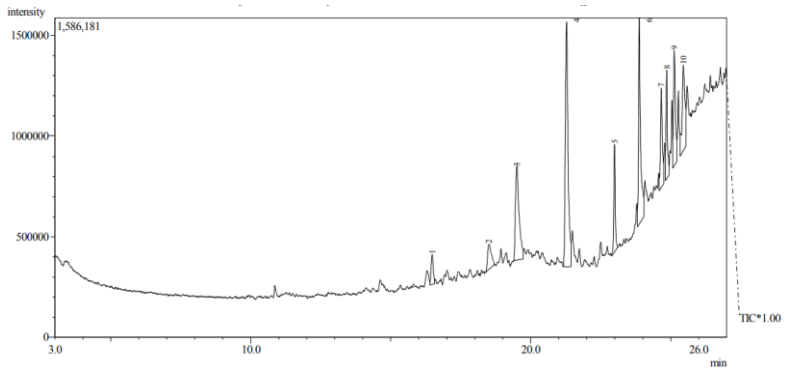

Fig. 1. GC-MS chromatogram of LME_3 fraction of *H. madgascariensis*

Table 5. Chromatogram showing the compounds of LME_3 fractions of *H. madagascariensis* leaf extract, retention time, concentration and name of compound

Peak	R. Time(s)	Area	Area (%)	Name of Compound
1	16.480	758588	2.53	Tetrahydro-alpha alpha-5-trimethy-5-vinyl furfuryl alcohol
2	18.510	1151508	3.65	3,4-altrosan
3	19.509	4291077	13.60	4-Hydro-N_2-(4,5,6,7 tetra Hydro-5-hydroxyminobenzofurazan -4-ylidino)-Benhydrazinde
4	21.282	8177932	25.92	2-(2-Hydroxy-2-phenyl ethoxy) Phenol
5	22.995	1898644	6.02	Methyldecanoate
6	23.878	5112506	16.20	n-hexadecanoic acid
7	24.661	2339011	7.41	(2E,4Z)-5-chloro-3,4-dimethyl-2,4 heptadiene
8	24.860	1994414	6.32	Methyl-11-octadecenoate
9	25.130	2756044	8.32	E,E-2,6-dimethyl-3,5,7-octatmene-2-ol
10	25.448	3032546	9.61	Oleic acid

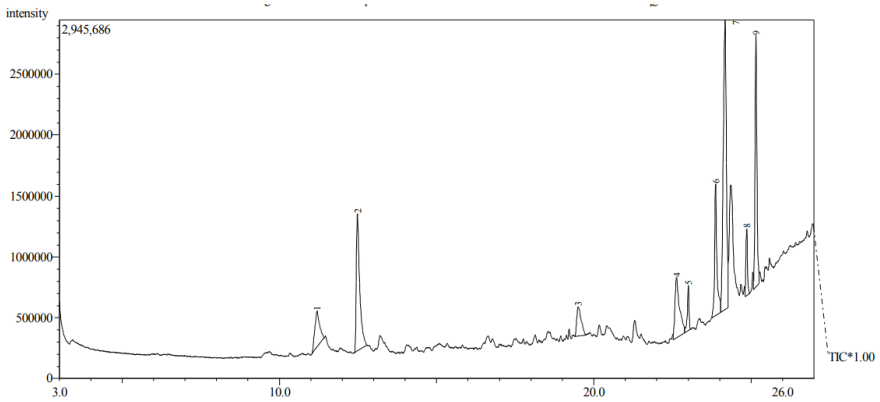

Fig. 2. GC-MS chromatogram of LME_4 fraction of *H. madgascariensis*

Table 6. Chromatogram showing the compounds of LME_4 fractions of *H. madagascariensis* leaf extract, retention time concentration and name of compounds

Peak	R. Time(s)	Area	Area (%)	Name of Compound
1	11.195	3104525	5.86	2,3-dihydroxyl propanal
2	12.481	8508229	16.07	Acetyl monoglyceride
3	19.499	2330851	4.40	4-Hydroxy benzoic acid
4	22.630	5005171	9.45	3-methyl-4-(phenylthio)-2-prop-2-enyl-2-5-dihydrothiophene-1, 1-dioxide
5	23.004	1381996	2.61	Methyl decanoate
6	23.871	5536340	10.46	n-octadecanoic acid
7	24.174	16378574	30.94	1-hydroxymethylcyclohexane (Nortricyclanol)
8	24.864	1927375	3.64	Cis-oleic acid
9	25.154	8770721	16.57	Z,Z-2,6-dimethyl-3,5,7-octattriene-Z-ol

Table 7. Chromatogram showing the compounds of LME_5 fractions of *H. madagascariensis* leaf extract, retention time, concentration (%) and name of compound

Peak	R. Time(s)	Area	Area (%)	Name of Compound
1	22.248	911394	6.75	Methylhexadecanoate
2	22.703	1269228	9.40	Methyl decanoate
3	24.804	11325608	83.85	Methyl-11-octadecanoate

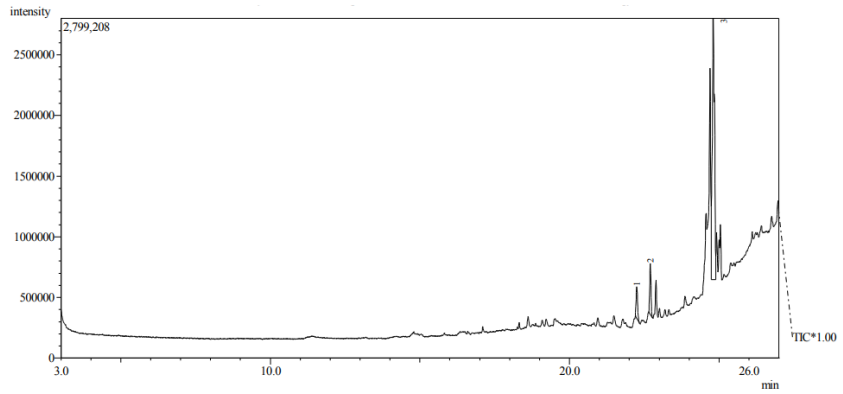

Fig. 3. GC-MS chromatogram of LME_5 fraction of *H. madgascariensis.*

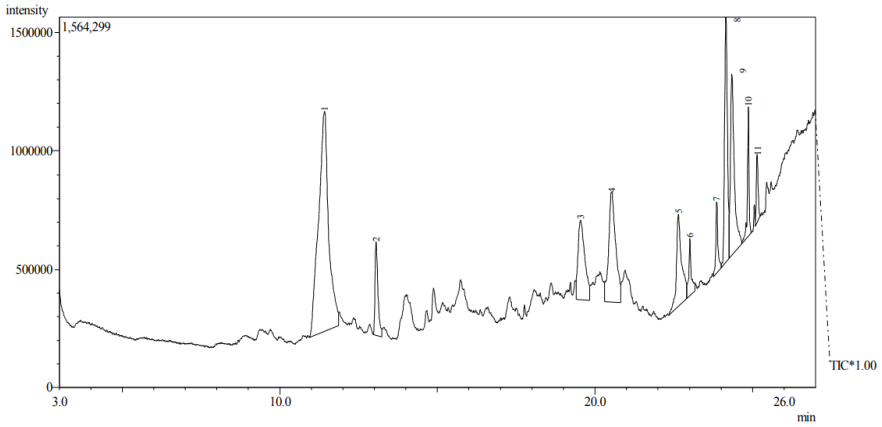

Fig. 4. GC-MS chromatogram of LME_6 fraction of *H. madgascariensis.*

Table 8. Chromatogram showing the compounds of LME_6 fractions of *H. madagascariensis* leaf extract, retention time, concentration (%) and name of compound

Peak	R. Time(s)	Area	Area (%)	Name of Compound
1	11.423	20770548	35.15	Glycerol
2	13.054	2608459	4.41	3,5-dihydroxy-6-methyl-2,3,dihydro-4H-pyran-4-one
3	19.540	5073295	8.59	4-Hydro-N_2-(4,5,6,7-tetrahydro-5-ylideno)-benhydrazide
4	20.515	7293784	12.34	3-deoxy-d-manonic acid
5	22.647	4516422	7.64	3-methyl-4-(phenylthio)-2-prop-2-enyl-2,5-dihydrothiophene-1,1-dioxide
6	23.010	1323991	2.24	Methyl decanoate
7	23.758	1399938	2.37	Decanoic acid
8	24.154	6598846	11.17	Nortricyclanol
9	24.342	6409615	10.85	10,13-dioxo-tricydo (6.3.3.0) tetradec-4-ene
10	24.869	1952126	3.30	Methyl-11-octadecanoate
11	25.144	1142431	1.93	Methyl Cis-2-(3-cyclo propyl-7-noncaranyl) acetate

Total of 31 fractions were collected from the column and they were pooled to six fractions according to their polarity. The details of fractions collected are given in Table 4. Hexane 100% and a mixture of hexane: chloroform at a ratio (50:50) did not elute much of the compound. The methanol extract fraction of *H. madagascariensis* was taken for bioassay and GC-MS analysis.

The bioassay of the pooled column chromatograph fraction revealed a favorable comparism in sensitivity between the control (ciprofloxacin) and LME_3 LME_4, and LME_6. While the diameter of the zone of inhibition of growth of *S. aureus* by the control (CPR) was 27mm, those of LME_3, LME_4 and LME_6 stood at 22mm, 20mm and 28mm respectively. This is in conformity with the reports that showed a minimum inhibitory concentration (MIC) value of 0.125 – 0.25 mg/ml for *S. aureus* [5]

Consequent upon the result of the bioassay, only LME_3, LME_4, LME_5 and LME_6, were taken for the GC-MS analysis. A total of twenty Seven (27) compounds were identified in the methanol fraction of *H. madgascariensis* leaf extract by GC-MS analysis. The chromatograms obtained as shown in Figs. 1, 2, 3 and 4, their corresponding retention time, area of peak, concentration (%) and name of compounds are presented in Tables 5, 6, 7 and 8 respectively.

The prevailing compounds were 2-(2-hydroxy-2-phenylethoxy) phenol, n-hexadecanoic acid, 4-hydroxy-N_2-(4,5,6,7-tetrahydro-5-hydroximinobenzofurazan-4-ylidino)-benzhydride, Nortricyclanol, acetylmonoglyceride, 3- methyl-4-(phenlthio) – 2-prop-2-enyl-2,5-dihydrothiophere 1,-1-dioxide, Z,Z-2,6-dimethyl-3,5,7-octatriene-2-ol Glycerol, 3-deoxy-d-mannonic acid, 10,13-dioxo-tricyclo (6:3:3:0) tetradec-4-ene and 3,4-altrosan. This study shows a very high percent of glycerol and 3,4-altrosan in the methanol fraction of *H. madagascariensis* leaf extract. Glycerol is taken orally for weight loss, improving exercise performance and helping the body replace water loss during diarrhea and vomiting. 3, 4 altrosan on the other hand, possesses bacteriostatic and fungicidal activity [14].

4. CONCLUSION

This study has revealed the presence of various therapeutically active compounds in the methanolic extract of *H. madagascariensis*. This further gives credence to its folkloric use in the treatment of microbial infections.

5. RECOMMENDATION

The authors wish to recommend that chromatographic analysis of the methanol extracts of stem and root be carried out in order to ascertain their phytochemical content being that they both inhibited the growth of the test organisms.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tupper KW. Psychoactive substances and the English Language: Drugs discourses, and public policy. Contemp Drug Probl. 2012;39(3):461-92.
2. Gill LS. Ethnomedical uses of plants in Nigeria. Nigeria: University of Benin press, Benin City. 1992;276.
3. Beentje H. Kenya trees, shrubs and lianas. National Museums of Kenya, Nairobi. 1994;721.
4. Agbor GA, Kuate D, Oben JE. Medicinal plants can be good sources of antioxidants: Case study of Cameroon. Pak J Biol Sci. 2007;10(4):537-44.
5. Okoro IS. Antimicrobial effect of the leaf extracts of blood tree (*H. madagascariensis*) on some human pathogens. J Med Appl Biosci. 2012;4.
6. Oboh G, Aomolafe TL, Adefega SA, Adetuyi AO. Antioxidant and modulatory of ethanolic extracts of *Harungana madagascariensis* Bark on cyclophosphamide induced Neurotoxicity in Rats. J Food Drug Anal. 2010;18(3): 171-9.
7. Kouam SF, Ngadjui BT, Krohn K, Wafo P, Ajaz A, Choudhary MI. Prenylated anthronoid antioxidants from the stem bark of *Harungana madagascariensis*. Phytochemistry. 2005;66(10):1174-9.
8. Tona L, Kambu K, Ngimbi N, Cimanga K, Vlietinck AJ. Antimicrobial and phytochemical screening of some Congolese medicinal plants. J Ethnophermacology. 1998;61:57-65.
9. Lukwa N, Mutambu SL, Makaza N, Molgaard P, Furu P. Perceptions about Malaria transmission and control using

antimalarial plants in Mola, Kariba, Zimbabwe. Niger J Nat Prod Med. 2001; 5:4-7.

10. Iwalewa EO, Naido V, Bagla V, Eloff JN. Nitric oxide producing effect of six extracts from *Harungana madagascariensis* com.ex poiret (gutteterae) stem bark. Afr J Pharm Pharmacol. 2009;3(1):014-21.
11. Brian KR, Turner TD. Practical Evaluation of Phytochemicals, Wright Scentechnical, Bristol, UK. 1975;57-59.
12. Trease GE, Evans WC. Pharmocognosy (Ed). Bailleiere Tindall East bourn. Pp (386-93)a, (395-398)b, (380-499)c, (500-517)d and (544-547)e; 2000.
13. Braithwaite A, Smith FJ. Chromatographic methods. 4th Ed. New York: Chapman and Hall Ltd. 1985;140-50.
14. Ravikumar VR, Gopal V, Sudha T. Analysis of Phytochemical Constituents of stem bark extracts of *Zanthoylum tetraspermum*. Arn. Res J Pharm Biol Chem Sci. 2012;3(4):391-402.

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