Volume 3 (1), 2015, Page-526-532
CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

PHS Scientific House
PHARMAHEALTHSCIENCES

International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Antioxidant Activity Among Selected Medicinal Plants Combinations (Multi-Component Herbal Preparation)

Oluwatosin Oladele Johnson *, Gloria Abiodun Ayoola
Department of Pharmaceutical Chemistry, University of Lagos, CMUL campus, Lagos, Nigeria.

ARTICLE INFO

Received: 11 Feb 2015
Accepted: 26 Feb 2015

ABSTRACT

Multiple-plant herbal preparations (multi-component herbal extracts) are used as remedies in many cultures around the world. In this study individual methanol plant extracts of the leaves of *Kigelia africana, Alafia bateri, Anthocleista djalonesis* and the stem bark of *Harungana madagascarensis* were screened for phytochemicals, free radical scavenging activity using 2,2-diphenyl-1-picrylhydrazyl and total phenolic content. These plants were selected based on their wide use by traditional medicine healers to treat various ailments in Nigeria. Different ratio combinations between *Harungana madagascarensis* and *Kigelia Africana* extracts and also for *Kigelia Africana* and *Alafia bateri* extracts were prepared and tested against free radicals. These individual extracts demonstrated anti-oxidant activity which was supported by the type of phytochemicals present in each extracts. The antioxidant and total phenolic content ranking order for the four plants tested were *Harungana madagascarensis* > *Kigelia Africana* > *Alafia bateri* > *Anthocleista djalonesis,* which corresponds to IC_{50} of 1.73µg/ml, 79.12µg/ml, 220.27µg/ml and 4704.62µg/ml respectively against ascorbic acid (1.38µg/ml) and vitamin E (6.39µg/ml), and likewise phenolic content of 84.4mg/g, 46.28mg/g, 43.82mg/g and 33.42mg/g respectively. The methanol preparation obtained from different ratio combinations of *Harungana madagascarensis* and *Kigelia Africana* suggests an overall reduction in scavenging activity of *Harungana madagascarensis*, with IC_{50} 90.80 µg/ml, 45.98 µg/ml and 52.16 µg/ml for 1:3, 1:1, 3:1 respectively, while the anti-oxidant activity of combinations between *Kigelia Africana* and *Alafia bateri* demonstrated antagonism, with IC_{50} 495.80 µg/ml, 1351.85 µg/ml, and 195.47 µg/ml for 1:3, 1:1 3:1 ratios respectively, which suggest they cannot be effectively used together to scavenge free radicals. **Keywords:** Antioxidant, multi-component herbal preparation, phytochemical screening, Total Phenolic content (TPC), 2,2-diphenyl-1-picrylhydrazyl (DPPH).

1. INTRODUCTION

Herbal medicine has become a popular form of healthcare, according to the World Health Organization (WHO); about 65-80% of the world's population which lives in developing countries depends essentially on plants for primary health care. [1]

Corresponding author *
O O JOHNSON*
Department of Pharmaceutical Chemistry,Faculty of Pharmacy, University of Lagos. CMUL campus, Idi-Araba. Lagos. Nigeria
E mail – : tosynvilla@yahoo.co.uk, tosyn.villa@gmail.com

In many societies around the world, multi-component herbal preparations have been used in the treatment of a wide range of diseases.[2] The concept of multi-component herbal therapy may be beneficial when the individual plants or plant parts extracts in the preparation possess different efficacies that provide additive or synergetic effects. It may also reduce the required doses of the individual components compared with mono-component herbal therapy and limit side effects.

On the other hand, some individual herbs in a multi-component preparation may have a negative effect on the overall efficacy of the multi-component herbal formulation due to masking and other chemical or physical interactions. In effect, herb-herb interactions in a multi-component herbal preparation may also result in antagonism. This area of study has attracted little attention in the scientific world, hence the need to conduct appropriate studies on possible interaction between different herbal preparations especially those that are often used together.

Kigelia africana (Lam.) Benth belongs to the family of Bignoniaceae and has a wide geographical distribution in west and central Africa. The tree grows on riverbanks, wet areas along streams and on floodplains of Nigeria, Cameroon, Kenya, Guinea and Senegal.[3] In South eastern Nigeria, the fruits and flowers are mixed with alcohol or water and used by traditional healers for fertility treatment among women and men of child bearing age. [4]

Anthocleista djalonensis A. Chev (Loganiaceae) is a medium-sized tree of the West tropical Africa, 30-45 feet high with blunt spines on the unbranch, pale grey trunk and widespreading crown. The stem, rootbark and leaves of *A. djalonensis* are used to treat malaria, jaundice, diabetes and abscesses.[5] The Ibibios of Southern Nigeria use the leaves and stembark as malarial remedy.[6]

Harungana madagascarensis Lan. ex poir is a species of flowering plants in the family of Hypericaceae and sole member of the genus Harungana. It is found in tropical Arica. The red juice from the leaves and the stem bark are used as anti-haemorrhage during childbirth, boiled water decoction of the root is used as an antidote in cases of liver and kidney poisonings while unopened buds are used in the treatment of skin diseases. [7, 8, 9.]

Alafia barteri (Apocynaceae) is a high-climbing, scandent shrub with small, pure white or pink flowers. [10] It is used in ethnomedicine for the treatment of sickle cell anaemia, rheumatism, eye infections, febrifuges, as chewsticks and toothache. The twining stem of *A. barteri* is used for the treatment of fever, inflammation and as binding materials for roots. [11, 12, 13.]

Therefore, the main objective of this study is to examine the anti-oxidant activities of two selected crude plant extracts at various volume ratio combinations. These results may provide an insight as to whether the extracts obtained from these various combinations will produce a more potent antioxidant effects than when used singly or an antagonistic effect, thereby serving as an indication to herbalist who specialized in mixture of two or more plants extract as remedies for various ailment.

2. MATERIALS AND METHOD

2.1 Plant materials

Apocynaceae, *Alafia barteri* Baker (Leaves), Loganiaceae, *Anthocleista djalonesis* A.cheu (Leaves), Bignoniaceae, *Kigelia Africana* Benth (Leaves), Hyperiaceae, *Harungana madagascarensis* Lan. ex poir (Stem Bark).

2.2 Collection and identification of plants

The plant materials were collect fresh from forest sources in Igbesa, Ogun state, South-west, Nigeria in March 2013, rinsed properly under running water and identified at Department of Botany, University of

Lagos. The plant Stem bark of *Harungana madagascarensis* were chopped into bits and air dried under shade for 21 days, while the leaves of *Alafia barteri, Kigelia Africana, and Anthocleista djalonesis* were also air dried under shade for 14 days. The dried plant materials were blended using a kitchen blender after which the powdered samples were weighed and was kept in small plastic containers in a cool dry place, with appropriate paper labeling.

2.3 Chemicals and reagents

In the course of the research work, the following analytical grade reagents were used: 2, 2-diphenyl-1-picrylhydrazyl, gallic acid, Folin–Ciocalteu reagent, ascorbic acid (vitamin C), Tocopherol Vitamin E, Sodium carbonate, Ethanol, Methanol, chloroform, sulphuric acid, acetic anhydride, 1% lead acetate, 2N Hydrogen chloride, isomyl alcohol, 10% sodium hydroxide, Ammonium hydroxide, ferric chloride, glacial acetic acid, Dragendoff, Wagner, and Picric acid reagent.

2.4 Extraction and Phytochemical of plants

The powdered plant material (100g) each for *Alafia barteri, Anthocleista djalonesis* and (150g) each for *Kigelia Africana, Harungana madagascarensis* were soaked in 1L 80% methanol for 72 h and the crude extracts were filtered through Whatman No 1 filter paper and concentrated using Rotary evaporator. Phytochemical screening was performed using standard procedures.[14]

2.5 Antioxidant activity of plant extracts

Determination of Total Phenolic contents (TPC).

The total phenolic content was determined according to a previously described method with slight modifications. Calibration curve was prepared by mixing ethanol solution of Gallic acid 1ml (25-400 µg/ml) with 5ml Folin-ciocalteu reagent (diluted ten folds) and Na_2CO_3 (4ml, 0.7M). Absorbance was measured at 765 nM and the calibration curve drawn. 1ml of ethanol plant extract (5mg/ml) was also mixed with the reagents above and after 2h the absorbance was measured to determine the total Phenolic content. All determinations were carried out in triplicates. The total Phenolic content in the extract in Gallic acid equivalents was calculated by the formula:

$$T = \frac{C \times V}{M}$$

Where T = total of Phenolic compound (mg/g plant extract in GAE).

C = concentration of Gallic acid established from the calibration curve (mg/ml).

V = Volume of extract (ml).

W = weight of plant extract (g).

Determination of free radical scavenging activity (FRSA) of plant extract.

The anti-oxidant activity of each extract was measured in terms of hydrogen donating or free radical scavenging activity, using the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). Briefly, to a methanolic solution (2 ml) of extract of various concentrations (20 – 100 µg/ml) was added 0.5 ml of 1 mM DPPH solution in methanol. A blank solution was prepared containing 2 ml of methanol and 0.5 ml of 1 mM DPPH. The experiments were carried out in triplicates. The mixtures in the test tubes were shaken and were allowed to stand for 15 min at room temperature, and the absorbance was read at 517 nm, methanol was used to zero the spectrophotometer. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The radical scavenging activity was calculated using the following formula:

$$\% \text{ inhibition of DPPH} = \{(A_{Blank} - A_{Sample})/A_{Blank}\} \times 100$$

Where A_{Blank} is the absorbance of blank sample (containing all reagents except the tested extract) and A_{Sample} is the absorbance of tested extract solution.

The results are expressed as percentage inhibition of DPPH and mean inhibitory concentrations (IC_{50}) determined from a plot of absorbance of DPPH versus extract concentration.

2.6 Interactions between plant extracts

Interactions between *H. madagascarensis* and *K. Africana*, / *A. barteri* and *K. Africana* against free radicals were determined at the corresponding fixed-ratio (1:3, 1:1, 3:1) combinations.

The IC_{50} values for the two different plant extracts (anti-oxidant action tested singly) were determined by the use of DPPH. (That was the result from the above FRSA determination).

The IC_{50} mix values for the corresponding fixed-ratio (1:3, 1:1, 3:1) antioxidant combinations was experimentally determined by the use of DPPH. IC_{50} mix is an experimentally determined total concentration of a mixture of two different plant extracts, which was used at a fixed-ratio combination, sufficient for 50% free radical scavenging activities. To determine the IC_{50} mix value, plant extracts in the mixture were tested for free radical scavenging activities using DPPH, and the results are expressed as percentage inhibition of DPPH and the mean inhibitory concentrations (IC_{50}) determined from a plot of absorbance of DPPH versus extracts concentration.

3. RESULTS AND DISCUSSION

The extraction procedure (cold maceration) gave a yield of 18.05 % for leaves of *Alafia barteri*, 16.26% for *Harungana madagascarensis*, 9.69% for leaves of *Anthocleista djalonesis* and *Kigelia Africana* 6.59 %yield. Phytochemicals such as flavonoids, tannins, and volatile oils were present in all the plant extracts (Table 1). Phytochemicals especially Flavonoids and polyphenols have received increasing attention because of their biological activities they constitute a major group of compounds that act as antioxidants. [15]

The DPPH test shows the ability of each plant extracts tested to act as a free radical scavenger. DPPH is a free radical and it produces a strong absorption band at 517 nm, in the visible region of the electromagnetic radiation. The colour turns from purple to yellow as the molar absorptivity of the DPPH reduces i.e. when the odd electron of DPPH becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. [16, 17] .The higher the % inhibition of DPPH absorbance the higher the FRSA and the lower the IC_{50} value the higher the FRSA/antioxidant power. Hence from this study, 80 % methanol extract of stem bark of *H. madagascarensis* (IC_{50} 1.73 µg/ml) had the highest FRSA compared to Vitamin E (6.89µg/ml) and the other plant extracts. Ascorbic acid had the highest IC_{50} (1.38 µg/ml) among all the compounds tested. The antioxidant ranking order for the four plants tested are *H. madagascarensis* > *K. Africana* > *A. bateri* > *Anthocleista djalonesis.*

Table 1: Phytochemical screening *Kigelia Africana, Anthocleista djalonensis,Harungana madagascarensis, and Alafia barteri.*

Phytochemicals	*Kigelia africana*	*Antholeista djalonesis*	*Alafia bateri*	*Harungana madagascarensis*
Flavonoids	+	+	+	+
Cardiac glycoside	-	-	-	+
Reducing sugar.	+	-	-	+
Alkaloids	+	+	+	-
Volatile oils	+	+	+	+
Terpenoids	+	-	-	+
Tanins	+	+	+	+
Anthocyanins	-	-	-	-
leucoanthocyanins	-	-	-	-
coumarin	+	+	-	+
Phenols	+	-	-	+
Saponin	-	+	-	+

Table 2: IC_{50} values, % Inhibition, Total Phenolic content for methanol plant extracts

Crude plant extracts	IC_{50} (DPPH Inhibition µg/ml.)	Antioxidant activity (IC_{50}(mean % Inhibition)	TotalPhenolic content (GAE) mg/gplant material.
Kigelia Africana.	79.12	40.08 ± 15.35	46.24
Anthocleista djalonesis.	4704.62	11.83 ± 5.48	33.42
Alafia barteri.	220.27	15.35 ± 5.90	43.82
Harungana madagascarensis.	1.73	94.49 ± 0.69	84.8
Ascorbic acid (Vitamin C).	1.38	97.17 ± 0.25	-
Tocopherol (Vitamin E).	6.39	88.69 ± 12.25	-

Table 3:Anti-oxidant interactions between *Harungana madagascarensis* (HM) and *Kigelia africana* crude (KA) plant extracts

Fraction HM : KA	IC_{50mix} (µg/ml)	Antioxidant activity (mean % inhibition)
1:3	90.808	34.30 ± 16.20
1:1	45.981	60.31 ± 23.85
3:1	52.168	57.46 ± 31.95

Table 4: Anti-oxidant interactions between *Alafia barteri* (AB) and *Kigelia Africana* (KA) crude plant extracts

Fraction KA : AB	IC_{50mix} (µg/ml)	Antioxidant activity (mean % inhibition)
1:3	495.80	6.96 ± 4.88
1:1	1341.85	7.51 ± 2.67
3:1	195.47	9.70 ±14.58

Plant phenolics are a major group of compounds acting as primary antioxidants or free radical scavengers. Therefore, it was reasonable to determine the total phenolic content in the plant extracts. The result of the total phenolic content ranking order for the different plant extracts are *H. madagascarensis* > *K. Africana* > *Alafia bateri* > *A. djalonesis* (Table 2). The graphical analysis showed a positive and strong relationship between the total Phenolic content and the antioxidant activity (r^2 = 0.905) suggesting that the antioxidant activity in these plants is largely due to the presence of phenolic compounds, especially for *H. madagascarensis* and *K. Africana*. (Fig 3). This shows that phenolics may be responsible for the FRSA (free radical scavenging activities) of these plants.

Results presented in this study indicate that the combination of *H. madagascarensis* and *K. Africana* for all fixed ratios (1:3, 1:1 and 3:1) tested, demonstrated a reduction in free radical scavenging activities of *H. madagascariensis*. Nevertheless, the IC_{50} mix for fixed ratio 1:1 and 3:1 (Table 3) may suggest that *H. madagascarensis* activity is potentiating the activity of *K. africana* in the mixture based on the IC_{50} of *K. africana* (79.12µg/ml) alone and IC_{50} for 1:1 (45.981µg/ml) and 3:1 (52.168µg/ml), but ratio 1:3 revealed that at a higher volume of *K. africana* and a much lower volume of *H. madagascarensis*, their interaction tends toward antagonism with IC_{50} 90.8 µg/ml.

However, the evaluation of the type of interaction between *K. africana* and *A. bateri* combination against DPPH free radicals at all fixed-ratios tested (1:3, 1:1 and 3:1), indicate antagonism. Thus, the individual plant extract may contain identical compounds and combining the plant extracts in a single preparation will merely increase their concentrations. The increased concentrations of the phytochemicals in the multi-component preparation may not have translated into an increased anti-oxidant effect.

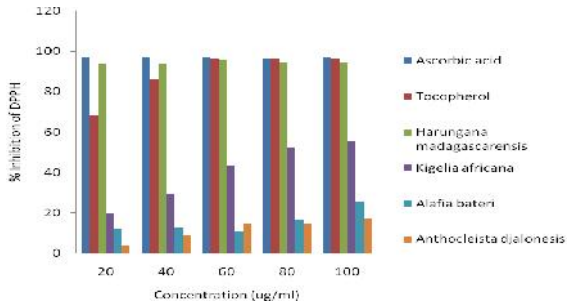

Fig 1: Inhibition (%) of DPPH against Concentration of extracts of *Kigelia Africana, Anthocleista djalonensis, Harungana madagascarensis, Alafia barteri* Ascobic acid and Tocopherol

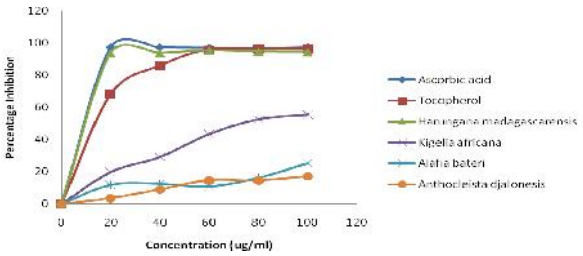

Fig 2: Graphical relationship between (%) Inhibition DPPH against concentration for the different crude plant extracts

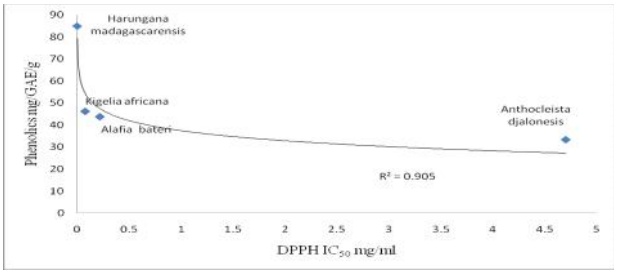

Fig 3.: Significant correlation between antioxidant activity and Phenolic content for the plant extracts.

4. CONCLUSION

The individual plant extracts of leaves of *Kigelia africana*, *Alafia bateri*, *Anthocleista djalonesis* and stem bark of *Harungana madagascarensis* were

screened for phytochemicals, free radical scavenging activity using DPPH and their total Phenolic contents were evaluated.

These extracts demonstrated anti-oxidant activity which was supported by the type of phytochemicals present in each extracts. The Antioxidant and Total Phenolic content ranking order for the four plants tested were *Harungana madagascarensis* > *Kigelia Africana* > *Alafia bateri* > *Anthocleista djalonesis.* Methanol multi-component preparation obtained from combination of *Harungana madagascarensis* and *Kigelia Africana* suggests a reduction in free radical scavenging activity of *Harungana madagascarensis*, which suggest that it should be used alone while the anti-oxidant activity of the multi-component combination between *Kigelia Africana* and *Alafia bateri* demonstrated antagonism, which suggests that they cannot be effectively used together to scavenge free radicals.

This also serves as an indication to herbalist who specialized in mixture of two or more plants extract as remedies for various ailments.

Isolation of active compounds from the mixture of the plant extracts will further explain the rationale behind the findings for future research.

5. ACKNOWLEDGEMENTS

We would like to appreciate Mr Adegoke, Mr I.O Olatunji and Mr. M. Olajide of Pharmaceutical Chemistry Department, University of Lagos, for excellent technical support.

6. REFERENCES

1. Akerele O. *WHO guidelines for the assessment of herbal medicines*. Fitoterapia 1992; 63: 99-104.
2. Huffman MA. Animal self-medication and ethno-medicine: exploration and exploitation of the medicinal properties of plants. Proc Nutr Soc. 2003; 62; 371–81.
3. Abioye AIR, Duru FIO, Noronha CC, Okanlawon AO. Aqueous extract of the bark of *Kigelia africana* reverses early testicular damage induced by methanol extract of *Carica papaya*. Niger. J. Health and Biomedical Sci. 2003; 2: 87-89.
4. Ogbeche KA, Ogunbiyi YO, Duru FIO. Effect of methanol extract of *Kigelia africana* on sperm motility and fertility in rats. Niger J Health and Biomedical Sci 2002; 2: 113-116.
5. J M Dalziel. The useful plants of West Tropical Africa. London: Crown Agents for Overseas Colonies. 1954.
6. Antia BS, Okokon JE, Etim EI, Umoh UF, Bassey EO. Evaluation of the in vivo antimalarial activity of ethanolic leaf and stembark extracts of Anthocleista djalonensis. Indian J Pharmacol. 2009; 41: 258-261.
7. Okoli AS, Okeke MI, Iroegbu CU, Ebo PU. Antibacerial activities of Harungana madascarensis leaf extracts. Phytother Res 2002; 16: 174-179.
8. Olagunju JA, Oladunni SO, Oladimeji MS. Status of phosphatase activities in the liver and kidneys of rat treated with isosaline leaf and stem bark extract of Harungana madacariences (L). Cytobiosis 2000; 103:17-24.
9. Iwalewa EO, Daniyan OM, Osoniyi OR Adetogun GE, McGraw LJ, NaidooV, Eloof JN. Mechanisms of the toxic effect of Harungana madagascariences stem bark in vivo and in vitro. J Trop Med Plant 2007; 8: 184-192.
10. Irvine F R. Woody Plants of Ghana, Oxford University Press. 1961; 736- 738
11. Leeuwenberg AJM. Series of revisions of Apocynaceae 43. Alafia Thouars. Kew Bulletin 1997; 52: 769-839.
12. Burkill H M. The useful plants of West Tropical Africa. 2nd Ed. Vol.1. Families, A-D. Royal

Botanic Gardens, Kew, Richmond, United Kingdom. 1985; 960.

13. Nadkarni A K. Dr. K.M. Nadkarni's India Materia Medica. With ayurvedic, Unani-tibbi, Saddha, allopathic, homeopathic, naturopathic and home remedies, appendices and indexes. Revised, enlarged and reprinted 1976; 3rd Ed. vol. 1&2. Bombay: Popular Prakashan.
14. Trease G E, Evans W C. Pharmacognosy. 11th edn. Bailliere Tindall, London, 1978; 176-180.
15. Cho EJ, Yokozawa T, Rhyu DY, Kim SC, Shibahara N, Park JC. Study on the inhibitory effects to Korean medicinal plants and their main compounds on the 1, LDPPH Radical. J Phytomedicine 2003; 10: 544-551.
16. Olaleye SB, Oke JM, Etu AK, Omotosho IO, Elegbe RA. Antioxidant and anti-inflammatory properties of a flavonoid fraction from the leaves of *Voacanga africana*. Nigerian Journal of Physiological Sciences 2004; 19: 69-76.
17. Yoshida T, Mori K, Hatano T, Okumura T. Studies on inhibition mechanism of autoxidation by tannins and flavonoids. Radical-scavenging effects on tannins and related polyphenols DPPH_ radical. Chemical and Pharmaceutical Bulletin. 1989; 37: 1919-1921.