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*Short communication*

# SCREENING OF SOME NIGERIAN MEDICINAL PLANTS FOR ANTIOXIDANT ACTIVITY USING 2, 2, DIPHENYL-PICRYL-HYDRAZYL RADICAL

***J.M. OKE; M,O. HAMBURGER**

*Department of Biological Pharmacy, Fried-Schiller University, Jena.Germany.*

*As part of a screening program for biologically active compounds in plants, twenty two medicinal plants were extracted and screened for anti-oxidant activity using the 2, 2,diphenyl-picryl-hydrazyl radical.*

***Key words**:- 2,2- diphenyl-picyl-hydrazyl, flavonoids, antioxidants*

**Dr. J.M. Oke was on study visit to Department of Biological Pharmacy, Fried-Schiller University, Jena. His present address is: Department of Pharmaceutical chemistry, University of Ibadan.*

## INTRODUCTION

Nature is and will still serve as the man's primary source for the cure of his ailments. However, the potential of higher plants as sources for new drugs is still largely unexplored. Consequently, in consultation with some prominent Nigerian native medicine practitioners, twenty two (22) Nigerian medicinal plant materials (Table 1) were selected for this study. These medicine practitioners have claimed that these plants materials are effective in the cure of central nervous system (C.N.S.) diseases e.g. psychiatric disorders, inflammation and pains. Recent interest in the study of antioxidants may not be unconnected with the efficacy of these compounds to cure most diseases of man particularly the C.N.S. ailments.

Antioxidants are radical scavengers which protect the human body against free radicals that may cause pathological conditions such as ischemia, anaemia, asthma, arthritis, inflammation, neuro-degenertion, Parkinson's diseases, mongolism, ageing process and perhaps dementias (Polterat 1997) Flavonoids and flavones are widely distributed secondary metabolites with antioxidant and antiradical properties. (Nakayoma and Yamada 1995).

This paper reports the phyto-chemical screening and isolation of the antioxidant components of these selected Nigerian medicinal plant materials.

## MATERIALS AND METHODS

### *Plant Materials*

All the plant materials (shown on Table 1) are collected around the Southern Western part of Nigeria and then their voucher specimen deposited at Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria.

### *Extraction Procedure*

50gm of the powdered specimen of each of the plant materials shown in Table 1 was extracted with 500mls of methanol for 3 days respectively. The coloured solution obtained from each of the plant material was concentrated by vacuum evaporation. The concentrate (extract) obtained was preserved for further use.

**Table1**

Plants tested for antioxidant activity

| Name | Family | Voucher No. |
|---|---|---|
| *Alstonia bonei*, cortex | Apocynaceae | 105370 |
| *Alstonia bonei*, folium | Apocynaceae | 105356 |
| *Alstonia bonei,* radix | Apocynaceae | 105350 |
| *Byrsocarpus coccineus*, folium | Connraceae | 105362 |
| *Byrsocarpus coccineus*, raidx | Connraceae | 105361 |
| *Cnestis ferruginea*, folium | Connraceae | 105373 |
| *Cnestis ferruginea*, lignum | Connraceae | 105357 |
| *Cnmestis ferruginea*, radix | Connraceae | 105375 |
| *Crinum purpurascens*, bulbus | Liliaceae | 105340 |
| *Crinum purpurascens*, folium | Liliaceae | 105341 |
| *Funtumia elastical*, folium | Apocynaceae | 105369 |
| *Hedranthera batteri* folium | Apocynaceae | 10563 |
| *Icacina trichantha*, folium | Icacinaceae | 105359 |
| *Icacina trichantha*, lignum | Icacinaceae | 105357 |
| *Icacina trichantha*, radix | Icacubaceae | 105360 |
| *Landolfia owariensis*, folium | Apocynaceae | 105368 |
| *Leea guinensis*, lignum | Leeceae | 105346 |
| *Leea guinensis*, radix | Leeceae | 105358 |
| *Sphenocentrum jollyanum*, folium | Menispermacea e | 105372 |
| *Sphenocentrum jollyanum*, radix | Menispermacea e | 105074 |
| *Voacanga africana* folium | Liliaceae | 105341 |
| *Canarium(spp),* cortex | Burseraceae | 105387 |

***Procedure***

1 mg of each extract was weighed into a small test tube and 10mls of methanol added. The mixture was shaken together and by use of capillary was spotted carefully on the aluminum-coated plate.

5ml of each of the mixture was spotted on the coated Aluminum plate about 10mm away from the bottom of the plate. The point of the spot was clearly labeled and the plate allowed to dry in air and developed in a tank containing the mobile phase: (Ethyl acetate: fomic Acid: water 85: 15: 10:)

The above was allowed to dry and viewed in the UV light at 365 and 254nm. The efflorescent points were marked at the wavelength after which the slide was sprayed with Diphenyl-Picryl-hydraxyl (DPPH) reagent in methanol (10mg in 10mls). After this, the plate was left to dry and the colouration produced on the plate was noted.

The DPPH reagent in this case was used to detect the presence of antioxidants. This reagent form complexes with the free hydroxyl group present in the crude extract. Examples of such compounds are the flavonoids, flavones etc. Thus DPPH on forming these complexes show the observed coloration (yellow coloration) on the TLC plate.

**RESULTS**

Table 2 shows the result of the anti-oxidant assay. All the medicinal plant materials used contain varying degrees of anti-oxidants. The colours of the efflorescence observed by the spots under the UV are a suggestion of the type of poly-phenolic compounds present in the sample (Wagner 1996). Although phytochemical screening showed that some of the plant materials contain other metabolites e.g. alkaloids, the major components contained in the plant material under investigation are poly phenolic compounds as confirmed by the D.P.P.H. spray reagent.

**DISCUSSION**

Flavonoids are groups of naturally occurring compounds widely distributed, as secondary metabolites in the plant kingdom. These Flavonoids have also been reported to possess anti oxidant and anti radical properties (Nakayoma and Yamada 1995).

The DPPH test (Wagner, 1996) provided information on the reactivity of test compounds with a stable free radical. Because of its odd electron, 2, 2- diphenyl-picryl-hydrazyl radical (DPPH) gives a strong absorption band at 517nm in visible spectroscopy (deep violet colour). As the electron becomes paired off in the presence of a free radical scavenger, the absorption varnishes, thus the resulting decolorization is stoictiometric with respect to the number of electrons taken up.

The scavenging properties of anti- oxidants are often associated with their ability to form stable radicals. Also, it is well known that aromatic compounds containing hydroxyl groups, especially those having ortho-di-or trihydroxyl functions can give rise to radical stable enough to be detected by ESR spectroscopy. The above is the general principle of the phytochemical screening for anti-oxidants.

**TABLE 2:**
Result of anti-oxidant assay

| Name of Medical plant | Result of antioxidant assay | Colour of efflorescent spot of the plant material |
|---|---|---|
| *Alstonia bonei*, cortex | ++ | |
| *Alstonia bonei*, folium | ++ | Bluish, yellow |
| *Alstonia bonei,* radix | ++ | |
| *Byrsocarpus coccineus*, folium | ++ | |
| *Byrsocarpus coccineus*, radix | ++ | |
| *Canarium(spp),* cortex | ++++ | Deep purple |
| *Cnestis ferruginea*, folium | ++++ | Yellow |
| *Cnestis ferruginea*, lignum | ++++ | Blue, yellow |
| *Cnmestis ferruginea*, radix | ++++ | Bluish yellow |
| *Crinum purpurascens*, bulbus | ++ | |
| *Crinum purpurascens*, folium | ++ | |
| *Funtumia elastical*, folium | +++ | Purple |
| *Hedranthera batteri* folium | ++ | |
| *Icacina trichantha*, folium | ++ | |
| *Icacina trichantha*, lignum | ++ | |
| *Icacina trichantha*, radix | ++ | |
| *Landolfia owariensis*, folium | +++ | Purple- bluish-purple |
| *Leea guinensis*, lignum | ++ | |
| *Leea guinensis*, radix | ++ | |
| *Sphenocentrum jollyanum*, folium | +++ | Orange, yellow |
| *Sphenocentrum jollyanum*, radix | ++++ | Orange, yellow |
| *Voacanga africana* folium | ++++ | Purple |

*+ + - antioxidant in low quantity; + ++- antioxidant in moderate quantity; ++++- antioxidant in large quantity.*

These spots are isolated by the preparative low pressure chromatographic method can be used for future structural elucidation of these

compounds by the help of spectrophotoscopic methods (Merbry *et al,* 1970). Diphenyl boryloxyl ethyl–amine is another regent similarly used in the laboratory to detect the presence of flavonoids in natural products. This method of screening also allows for a bio-assay guided study of natural products.

This study reveals the presence of antioxidant activity in varying degrees in all the plant materials under study. Bio-assay guided studies on all these medicinal plants and their structural elucidation of the active isolates are progressing in our laboratories.

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## REFERENCES

**Marbry T.J.K.B. Markham and M.B. Thomas (1970),** The Systemic identification of flevonoids Published by Spinger-New York.

**Nakayoma, J and Yamada M. (1995)** Suppression of active oxygen-indeed cyto toxicity by flavonoids. Biochem. Pharmcol. 45; 265-267.

**Polterait O. (1997)** Anti Oxidants and free-radical Scavengers of Natural origin Current Org. Chem. 1.415-440

**Tomaturo Takao (1994)** A simple screening method for anoxidants and isolation of several anti oxidants produced by marine bacteria from fish and stellfish J. Biosci, Bis tech. Biochem. 58 (10) 1780-1783.

**Wagner S. (1996).** Plant Drug analysis - a thin layers chromatogratlas. 2nd Ed. Springe. Pg. 195 – 197, 359 – 364.

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