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Antioxidant potentials of five plants used in Akwa Ibom state Ethnomedicine for Pain

Uwemedimo Umoh, Paul Thomas, Emmanuel Etim, Imo Jacobs and Emmanuel Bassey

Abstract
Five plants *Alstonia boonei* (leaf), *Ficus exasperata* (leaf), *Nauclea latifolia* (leaf), *Raphia hookeri* (root) and *Vernonia amygdalina* (leaf) used in Akwa Ibom State of Nigeria ethnomedicine for the treatment of pain associated diseases were studied for their antioxidant properties using total flavonoid contents, rapid radical scavenging assay and diphenyl-1-picryhydrazyl (DPPH) assay models. Results of the study revealed a positive correlation in the total flavonoid contents with *Raphia hookeri* showing the highest value of 50 $\pm$ 00 in garlic acid equivalent and *Ficus exasperata* being the lowest with a value of 30$\pm$00 in gallic acid equivalent. The plants displayed potent DPPH scavenging potential with *Raphia hookeri* still being the highest and *Ficus exasperata* the lowest. Ultraviolet spectroscopic evaluation showed a dose-dependent scavenging of DPPH radical to non-radical forms in all plant extracts. The results of this study support the use of these plants in the management of pain related disorders.

Keywords: Antioxidant, ethnomedicine, plant-extracts, pain

1. Introduction
Akwa Ibom State is one of the states in Southern Nigeria with rich diversity of medicinal plants that have been implicated in the management of many diseases associated with pain) [1, 2]. *Raphia hookeri* G. Mann & H. Wendi (Arecaceae) commonly known as Raphia palm wine, palm is a monoecious tree found from Gambia through the Guinea forest zone of West Africa and the juice from it is drunk for its high yeast content and refreshing taste and the root decoction is used for management of inflammatory disorders by the Ibibios of Akwa Ibom State among other uses; *Alstonia boonei* De Wild (Apocynaceae) is a large deciduous tree native to tropical west Africa whose stem bark is a remedy in Akwa Ibom State ethnomedicine for expulsion of intestinal worms, reduction in filarial-induced swelling, treatment of malaria, yaws, gonorrhea, sores, rheumatic pain and toothache [1]; *Vernonia amygdalina* Delile (Asteraceae) is a common vegetable whose leaf decoction is used to treat fever, malaria, diarrhea, cough, diabetes and as worm expellants [1, 3]; *Ficus exasperata* Vahl (Moraceae) commonly known as sand paper tree, fig tree is a terrestrial afro-tropical shrub or small tree used by the locals of Akwa Ibom State for the treatment of fever, ulcer and dental caries[1] While *Nauclea latifolia* L. (Rubiaceae), a straggling of scadant or small spreading tree has been implicated in the treatment of malaria, stomachache and as antidepressant[1].
The decision to investigate these plants was to establish whether their uses in the management of pain disorders are related to their anti-oxidant potentials.

2. Materials and Methods
2.1 Collection and Identification of plants: The leaves of *A. boonei, F. exasperata, N. latifolia, V. amygdalina* and the root of R. *hookeri* were collected from Afaha Oku village in Uyo Local Government Area, Akwa Ibom State, Nigeria and authenticated by Dr. (Mrs.) Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo, and Herbarium Specimens deposited in the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo, Nigeria.

2.2 Preparation of Extracts: The collected plant parts were garbled, air-dried and powdered. The powdered plant materials (500g) each were macerated in 50% ethanol for 72 hours. The liquid extracts were concentrated to dryness in *vacuo* at 40 ^{0}C and stored in a refrigerator from where they were being used for the various analysis.

2.3 Estimation of Total Flavonoid Contents

The method of Meda *et al*., (2005) [4] was adopted for this study. 5ml of 2% Aluminium trichloride ($AlCl_3$) in methanol was mixed thoroughly with the same volume of extract solutions. Absorption readings at 517nm using UV-visible spectrophotometer were taken after 15minutes against a blank sample consisting of a 5ml crude extract and 5ml of the different extracts with 5ml methanol without $AlCl_3$. Total flavonoid content was determined using a standard curve prepared with garlic acid (0.1 – 0.8mg/ml) and expressed in mg/GAE/lg of extract.

2.4 DPPH Rapid Scavenging Test

This study was done according to the method of Adebajo *et al*., (2009) [5]. Stock solutions (1.0mg/ml) of the extracts at various concentrations and ascorbic acid were spotted on a silica gel thin layer chromatographic plate using capillary tubes. The plate was developed in a solvent system (ethanol 90: methanol 10) and the chromatogram was dried and sprayed with 0.3mM solution of DPPH. The duration for the development of yellow color was noted and this was taken as an indicator for antioxidant activity [6]

2.5 Diphenyl-l-Picrylhydrazyl (DPPH) Assay

The method of Blois (1985) [6] was adopted for the study. 5ml of various concentrations (0.2 – 1.0mg/ml) of plant extracts and ascorbic acid were added to 1.0ml of 0.3mM DPPH in methanol. The mixtures were vortexes and incubated in a dark chamber for 30minutes after which the absorbance were measured at 517nm using UV-visible spectrophotometer against a DPPH control containing only 5ml of methanol. Percentage scavenging activity was calculated thus:

$$\% \text{ Scavenging Activity} = \frac{\text{Absorbance of control} - \text{Absorbance of Extract}}{\text{Absorbance of control}} \times 100\%$$

The concentrations of extracts that gave 50% inhibition of DPPH (IC_{50}) were obtained from the graph of percentage inhibition versus concentration in µg/ml [7].

3. Results

Table 1: Result of Phytochemical Screening of Ethanol Plant Extracts

Chemical constituents	Test	*A. boonei* leaf	*N. latifolia* leaf	*V.amyygdalina* leaf	*F. exaperata* leaf	*R. hookeri* leaf
Alkaloids	Dragendorff's Test	+	+	-	-	+
	Mayer's Reagent	+	+	-	-	+
Saponins	Frothing Test	+	+	+	+	+
	Sodium Bicarbonate	+	+	+	+	+
Tannins	Ferric Chloride	+	+	+	+	+
	Bromine Water	+	+	+	+	+
Terpenoids	Tetraoxosulphate (vi) Acid Test	+	+	-	-	+
	Acetic Anhydride	+	+	-	-	+
Cardiac Glycosides	Salkowsky's Test	+	+	-	+	+
	Keller-Killiani Test	+	+	-	+	+
	Lieberman's Test	+	+	-	+	+
Flavonoids	Magnesium metal	+	+	+	+	+
	$ALCl_3$	+	+	+	+	+

Note: + present, - absent

Table 2: Result of Total Flavonoid Contents of Plant Extracts

Extracts	Total Flavonoid Contents mg/GAE	λ max(nm)
V. amygdalina	32.5	0.228
F. exasperate	30.0	0.214
A. Boonei	40.0	0.234
N. latifolia	37.0	0.219
R. hookeri	75.5	0.305

Table 3: Result of DPPH Rapid Scavenging Test of Plant Extracts

Extracts	Reaction speed
V. amygdalina	Fast
F. exasperate	Moderate
A. boonei	Fast
N. latifolia	Fast
R. hookeri	Very fast

Table 4: Result of DPPH Assay of extracts at 517nm expressed as percentage inhibitions

Concentration (mg/ml)	*V. amygdalina* l	*F. exaperata*	*A. boonei*	*N. latifolia*	*R. hookeri*	Ascorbic Acid
0.4	36	35	36	35	35	91
0.6	56	42	57	43	49	91
0.8	77	56	78	56	56	91
1.0	78	77	79	56	80	92

4. Discussion

When reactive oxygen species (ROS) are produced beyond what the body can handle, oxidative stress and damage occur which results in disorders such as diabetes, arthritis, cancers and many other human diseases. Antioxidants are known to protect the body by combating oxidative damage [8]

The result of phytochemical screening of these five plant extracts (table 1) revealed the presence of saponins, tannins, flavonoids, alkaloids, terpenoids and cardiac glycosides in varying amounts in *A. boonei* and *N. latifolia* while terpenoids were not present in *V. amygdalina, F. exasperata* and *R. hookeri*. Alkaloids were also not present in *V. amygdalina* and *F. exasperate*.

The results of total flavonoid contents of the ethanol extracts (table 2) expressed in mg/GAE are 40.0, 75.5, 32.5, 30.0 and 37.0 for *A. boonei, R. hookeri, V. amygdalina, F. exasperata* and *N. latifolia* respectively. Many researchers have established a direct correlation between antioxidant activities,

flavonoid contents and the potency of plants in their uses in the management of debilitating disorders [9].

The result of DPPH rapid scavenging test for the five plant extracts (table 3) showed that *R. hookeri* root ethanol extract reacted very fast with a very high spot intensity followed by *N. latifolia* leaf extract; *A. boonei* leaf extract and *V. amygdalina* leaf extract with fast spot intensity and finally *F. exasperata* with moderate reaction speed. This result could be viewed from the total flavonoid contents of these extracts. *R. hookeri* root extract had the highest flavonoid content of 77.5 with *F. exasperata* having the least 30.0. A higher flavonoid content here means a fast reaction speed while a low flavonoid content means a slow reaction speed as demonstrated by these plants extracts.

The result of the DPPH assay of the extracts at 517nm expressed as percentage inhibition at 1.0mg/ml (table 4) presented *R. hookeri* 80, *A boonei* 79, *V. amygdalina* 78, *F. exasperata* 77 and *N. latifolia* 56 in decreasing order of percentage inhibition with ascorbic acid, a standard antioxidant being more potent than these plants extracts with a percentage inhibition of 92 at 1.0mg/ml concentration. The ability of these plant extracts to exhibit high percentage inhibitions may also in part be related to their flavonoid contents [10]. The use of these plants in the management of pain related disorders may not be unconnected to their phenolic contents [11].

5. Conclusion

The results of this study support the various uses of these plants in Akwa Ibom State ethnomedicine for the management of arrays of pain related disorders.

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