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Phytochemical Screening And Analgesic Studies Of The Root Bark Of Hymenocardia Acida, Tul (Euphorbiaceae)

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Abstract

The research work covers the phytochemical screening and Analgesic studies of the root bark of *Hymenocardia acida,* Tul, (Euphorbiaceae) which is claimed by the Hausa in the Northern Nigeria to be used traditionally for the treatment of headache, chest-pain, rheumatic pain, toothache, ear pain, migraine and sickle cell crisis. The various phytochemical tests revealed the presence of carbohydrates, tannins, alkaloids, flavonoids, saponins and cardiac glycosides. The result of analgesic activity of the extracts showed a significant and dose dependent analgesic activity when compared to the untreated control group at P<0.05. This justifies the use of the plant in ethno-medicine for the treatment of headache, chest pain, rheumatic pain, toothache, ear pain, migraine and sickle cell crisis.

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Key words:
Hymenocardia acida, root bark, analgesic, chromatography

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Introduction

The plant *Hymenocardia acida* is a tree of about 6 m high, gnarled and twisted with characteristics rough rusty-red bark, of the wooden savanna throughout the region from Senegal to west Cameroons, and widespread in tropical Africa. The wood is light brown or pink, darkening to orange, close- grained, with conspicuous annual rings, and hard. It has been

said to be brittle and good only for firewood. This may be reflected in certain Ivorian names meaning 'The tree which kills the wife', i.e. when an unfaithful wife goes to collect firewood, the tree shatters at her tough and a branch pierces her abdomen. [1].

The Gbaya of the Central African Republic recognize the tree as producing good firewood; indeed classified it as 'woman's firewood' being good for the hearth and cooking place, long- lasting while the house wife is about other chores, yet reviving quickly from sleeping embers, with a hot flame and little smoke [2]. The tree is used for house- posts in southern Nigeria, and in Gabon where the wood is made into charcoal for blacksmith's work. In Kenya and in Uganda, the wood is known for its hardness, denseness, durability and good resistance to termite-attack. It is used to make pestles and back- cloth mallets. Charcoal made from the branches is powdered and rubbed on the head for headache in the soudanian region [3].

The foliage is browsed a little by cattle in Senegal as a supplementary food and in Nigeria. Leaves and leafy shoots have a considerable medicinal use. When chewed, they have an acid taste. Leaves are prepared into infusion for use in Senegal for chest- complaints and small- pox and with the roots, for deficiency diseases. A macerate is given for gripe, and leaf-decoction used as an eye wash [4]. A decoction with honey is taken in Guinea for biliousness. In Ivory coast- upper Volta, a leaf- decoction is used in baths and draughts as a febrifuge and leaf- powder is taken as snuff for headache or applied topically for rheumatic pains and toothache, or for the same purposes, leaves may be pulped with an organic acidic substances such as citron juice or sap of Piliostigma reticulatum (leguminosae: caesalpinioideae)[1].

The Hausa tribe in the Northern Nigeria use Hymenocardia *acida* for the treatment of headache, chest pain, rheumatic pain, toothache, ear pain, migraine and sickle cell crisis when the decoction of the leaves and the stem bark or the root bark is combined together. Also in Senegal, the root- bark alone is extracted and used for the treatment of toothache and in Congo for earache when it is applied topically.

This study aims at investigating the general pharmacognostic and phytochemical profile of the root bark and to carryout analgesic studies and also Provide a monograph on the plant to justify the use of the plant in traditional medicine.

MATERIALS AND METHODS

Plant Collection and Identification:

The root bark of *Hymenocardia acida* was collected from Kudingi village, Zaria, Nigeria on the 15th of January, 2010. The plant was identified in the field using keys and description given in the official books [5,6]. The collection was confirmed and authenticated at the Herbarium, Biological Sciences, Ahmadu Bello University, Zaria and the voucher number, 1010 given.

Preparation of Extracts:

The fresh root bark of *Hymenocardia acida* was air dried, powdered, sieved and referred to as 'powdered root bark'. The powdered root bark (500g) was defatted exhaustively with petroleum ether (60-80^{OC}) in a soxhlet apparatus. The defatted marc was subsequently extracted with 70% methanol. The extract was concentrated in- vacuo and produced a dark brown mass (50g) subsequently referred to as the 'methanol extract'. The fractionation process was carried out as given on scheme1 below. The different fractions were subjected to various tests for the presence of phytochemical constituents and analgesic properties. The residues were dissolved in their respective solvent used for their fractionation.

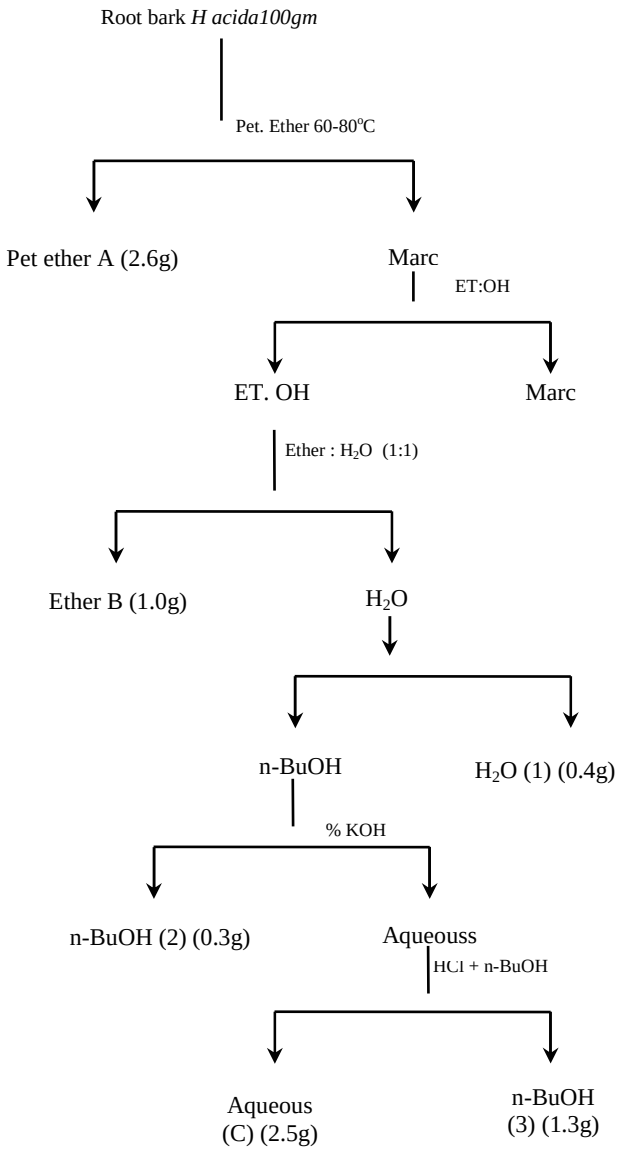

Fig 1: Extraction of the root bark of *Hymenocardia acida*. *Key: pet. ether- petroleum ether, H_2O – water, Et.OH- ethanol, n- BuOH- n- butanol, HCl- hydrochloric acid.*

Phytochemical Screening

The methanol extract and the different fractions of the root bark was subjected to phytochemical screening for the presence of chemical constituents such as alkaloids, saponins, flavonoids, tannins, cardiac glycosides, anthraquinones and carbohydrates according to standard procedure [7].

Analgesic studies

(Acetic acid- induced abdominal constriction in mice):

The method described by Koster *et al.* [8] was used. The Mice were grouped into 10 each containing 5 animals. Group 1 were given acetic acid (0.7%v/v in saline) intraperitoneally, Ip. Group 2 and 3 were given paracetamol, 500mg and 1000mg Ip before Acetic acid injection. Group 4, 5, 6, 7, 8 and 9 (Test groups) were given *Hymenocardia acida* extracts (sample 1, 2 and 3) as indicated in scheme 1 at doses of 50mg and 100mg 30 minutes before acetic acid injection. Group 10 were given 0.1ml Normal saline 1p. The number of abdominal constrictions produced in each group for the succeeding 5 minutes was counted and compared with the response of other groups. This was calculated as the percentage of inhibition of abdominal constrictions.

RESULTS

Table 1: Phytochemical Constituents H. *Acida*

Phytochemical Test	Root-bark
1. CARBOHYDRATE TEST	
Molish's test	+
Barfoed's test	-
Fehling's test (reducing sugars)	+
Fehling's test (combined reducing sugars)	+
Ketoses test	+
Pentose test	-
2. TANNIN TEST	
Pd Subacetate	+
$FeCl_3$	+
Formaldehyde/HCl	-
Bromine water	+
Lime water	+
3. GLYCOSIDES TESTS	
Bontrager's test	-
Haemolytic test	+
Keller-Killiani test	+
Kedde test	+
Froth test	+
Cyanogenetic glycosides	-
4. FLAVONOIDS TEST	
Lead acetate test	+
NaOH (20%)	+
$FeCl_3$	+
Shinoda test	+
5. TERPENES/STEROLS	
Salkowski's test	+
Liebermann-burchard's test	+
6. RESINS/BALSAM	
CU-acetate	-
Acetic hydride and conc. H_2SO_4	-
7. TESTS FOR ALKALOIDS	
Mayer's test	+
Dragendoff's test	+
Wagner's test	-

Key: + Present; - Absent

Table 2: Phytochemical Screening of the Fractions of *H. acida*

Sample	Heamolysis Test	Pb. Acetate Test	Pb. Subacetate Test	Mayer's Reagent	$Fecl_3$ Test	Inference
1	+	+	+	-	+	Phenolic compounds & Saponins
2	+	-	-	-	-	Saponins
3	+	-	-	-	-	Saponins
C	-	+	+	-	+	Phenolic compounds only

Key: + Present; - Absent

Table 3: Results of TLC Analysis of the Fractions of Hymenocardia acida Root-bark.

Samples	Rf Value	Dragendorff's Spray Reagent	Inference
1	0.00 0.12	Yellow Orange	- +
2	0.11 0.00	Yellow Brown	- -
3	0.13 0.30	Orange Yellow	+ -
C	0.14 0.00	Orange Brown	+ -

Key: + Present; - Absent

SOLVENT SYSTEM: Chloroform : Methanol (2:3)
Adsorbent : Silica gel.

Table 4: TLC Results of the Alkaloidal Extracts of the Root-Bark of Hymenocardia acida

Extract	Rf-value	Day Light	UV254nm 366MN	After Spray (Dragendoff's)
1*	0.34	Visible	No fluorescence	Pale orange
2*	0.11	Visible	No fluorescence	Orange
3*	0.21	Visible	No fluorescence	Brown
4*	0.31	Visible	No fluorescence	Brown
5*	0.40	Visible	No fluorescence	Pale orange
6*	0.50	Visible	No fluorescence	Brown
7**	0.60	Visible	No fluorescence	Brown
8**	0.73	Visible	No fluorescence	Orange

Key: + Present; - Absent

SY 2: Chloroform : Methanol 9:1
SY1: Chloroform : Methanol 3:2

EVALUATION OF THE ANALGESIC ACTIVITY OF THE EXTRACTS

The percentage inhibition of the abdominal constrictions with sample 1, 2 and 3 was significant (at P< 0.05) using one way ANOVA. This is to prove that the fractions contain phenolic compounds, saponins and alkaloids and so have analgesic properties. The most active been the saponins (samples 2) and alkaloids (samples 3), followed by phenolic compounds and saponins (sample 1) and then phenolic compounds only (sample C). It is to be noted it is only TLC that detected the presence of alkaloids in sample 3. The phytochemical screening gave a negative result.

DISCUSSION

Phytochemical screening revealed the presence of alkaloids, saponins, flavonoids, tannins, cardiac glycosides and anthraquinones. The chromatographic results further indicated the presence of alkaloids which is in agreement with the phytochemical tests. The presence of alkaloids was not detected in the phytochemical screening of the fractions but seen with TLC because; chromatography is a more sensitive method.

The chemical constituents were responsible for the pharmacological actions of the root bark. Sample 1, 2 and 3 showed analgesic properties. Alkaloids, flavonoids and saponins are known to possess analgesic activity [7]. The activity of the extracts was found to be dose dependent and significant at P<0.05. Data analysis of the analgesic activity further indicated that, saponin extract exhibited a very significant reduction in pain. This means that, the extract exhibited a very significant analgesia.

Comparison of analgin® at different concentration showed no significant effect. This indicated that the analgesic property of these extracts is not as effective as analgin®.

CONCLUSION

The analgesic activity of the extracts justifies the traditional use of the plant in the treatment of pain.

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