Elucidation and Physicochemical Analysis of Bioactive Composition of Leaves and Root Methanol Extract of Robinia Pseudoacacia (Black Locust)

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Abstract- ***The Elucidation and physicochemical analysis of bioactive composition of leaves and root methanol extract of Robinia pseudoacacia (black locust) is carried out successfully. All chemicals and reagents used in this study were of analytical grade. Mean Proximate Composition for both the leaves and the root gives (%)Protein14.88±0.09, 20.91±0.18; (%)Ash 2.71±0.02, 1.94±0.09; (%)Moisture 28.28±0.51, 19.81±0.13; (%)Fats22.13±0.05, 10.06±0.25; and (%)Fibre 0.28±0.04, ND. Mean Phytochemical Composition for both the leaves and the root respectively gives (%)Saponin 5.54±0.13, 0.11±0.16; (mg/g)Flavonoids 5.73±0.09, 1.87±0.02; (µg/g)Steroids 33.64±0.22, 27.98±0.35; (%)Alkaloid 39.39±0.86, 8.00±0.62; (%)Tannin 0.30±0.01, 0.22±0.002; and (mg/100g)Glycoside 65.11±1.39, 27.54±0.23. Acute toxicity test was carried out on both leaves and root extract of the plant. For both Stage 1 and Stage 2 of the test, none of the animals died and there were no signs of intoxication in all the mice. Chronic toxicity test was also done for both leaf and root extracts and the results showed no sign of intoxication in the tested mice. Functional groups composition of the Robinia pseudoacacia was also determined. Both the leaves and roots moderately contain alkene groups. The leaves have more phenolic and amino groups than that of the roots. Carboxylic acid is contained in both the leaves and the roots but it is more pronounced in the leaves. There is no alcoholic group in the leaves but it is present in the root at a very low concentration. Aldehyde group is present in the leaves but absent in the roots. This research revealed that the Robinia pseudoacacia (Black Locust) tree grown in the eastern part of Nigeria is not toxic.***

Indexed Terms- ***Robinia pseudoacacia, physicochemical, bioactive compound, Acute Toxicity, Chronic Toxicity***

I. INTRODUCTION

Black locust is a perennial shrub or medium-sized tree. Each compound leaf has 7 to 25 leaflets arranged such that one leaflet is located at the tip and the rest are in pairs. At the base of young leaves are two spines, one located on each side of the leaf stalk (petiole) where it attaches to the stem. Other characteristic features of this member of the bean family include fragrant white flowers in long, drooping clusters and 4-inch-long, brown seedpods. Reproduction is by seeds and shoots that sprout readily from roots of established plants.

The scientific name of black locust is *Robinia pseudoacacia* (Diggs, 1999). Black locust matures to a medium-sized tree, generally 40 to 60 feet (12-18 m) in height and 12 to 30 inches (30-76 cm) in diameter (Huntley, 1990). Young black locust bark is smooth and brown. Young trees are thorny. As trees age, the bark becomes thick, deeply furrowed, scaly, and dark brown. Black locust leaves are deciduous, alternate, and pinnately compound, with 7 to 19 leaflets on a central stalk that is 8 to 12 inches (20-30 cm) long. Leaflets are 30 to 50 mm long. Black locust flowers are showy, white, and fragrant, in drooping clusters about 6 inches (14 cm) long. Clusters arise from leaf axils near the tip of new shoots. Black locust fruits are flat legumes 3 to 4 inches (7-10 cm) long. Seeds are dark, bean-like, 3 to 5 mm long, and have a hard, impermeable coat. Each legume contains approximately 4 to 8 seeds (Laird, 1995).

Black locust is poisonous to all animals if ingested. Although fatal cases are rare, recovery may take

several days or even weeks. There have been reports of children poisoned by chewing the inner bark or eating seeds. However, most reported cases involve horses that became ill after eating young shoots or chewing bark. Cows, chickens, and sheep have also been poisoned. The toxic young shoots appear to be desired by livestock, even if there is plenty of other forage available (Ohio Agricultural Research and Development Centre).

Background of Study

Black locust (*Robinia pseudoacacia*) was the first North-American tree species imported to Europe at the beginning of the seventeenth century. It is commonly planted worldwide because of its adaptability to environmental stresses. The properties include valuable wood, easy propagation, frequent and abundant seed production, high seedling survival, and relatively high wood yield. In Europe, Romania and Hungary have the most highly-developed black locust growing techniques and experiences (Nicolescu VN, March, 2018).

Black locust (*Robinia pseudoacacia*) originated in the eastern part of North America, particularly in the Appalachian regions. By the early 17th century, black locust had been introduced to Europe. Today, black locust appears in many European countries, especially in Hungary, Northern Germany, Western Poland, Czech Republic, Southern Slovakia, Eastern Austria and Africa.

Black locust (*Robinia pseudoacacia*), a tree of North American origin, is the most common alien woody species in the Czech Republic, dominating in 0.6% of the total forested land (Úhúl 2007). It was extensively planted in the nineteenth century in warm areas as a source of honey, hard, resistant wood and for reducing soil erosion (Nožička 1957; Vítková et al. 2017).

Drought makes *R. pseudoacacia* useful for technical reclamation of N- deficient post-industrial sites, but problematic for nature conservation (Rice et al., 2004; and Vítková et al., 2017). It was proved that the invasion of *R. pseudoacacia* into nutrient-poor, pine-oak ecosystems elevated nitrogen, phosphorus, and calcium (N, P, Ca) concentrations, net nitrification and N-mineralization (Nitrogen-mineralization) rates in soil, which was associated with greater litter fall mass as well as high N (nitrogen) and low lignin content in *R. pseudoacacia* leaf litter (Rice et al., 2004). Black locust *(Robinia pseudoacacia)* provides wood, honey and cultural services.

II. MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used in this study were of analytical grade and were products of May and Baker, England, Merck, Germany, Sigma – Aldrich, Germany, British Drug Honses (BDH), UK and Kieselgel GmbH, Germany, Reagents used for the assays were commercial test Kits and products of Randox, UK, Biovendor, Czech Republic, TECO Diagnostics, USA and Centronic GmbH, Germany, dutasteride (Avodart).

Equipment

The equipment used are: Rotary evaporator, (Model 349/2, Corning Ltd, England); Centrifuge (Gallenkamp, Germany); Spectrophotometer (Jeol 400 MHz, Strathclyde Scotland University); glass column chromatography (Pyrex, England); vacuum pump; milling machine; filter paper; digital photo colorimeter; Olympus microscope; water bath (Chikpas Instrument, Enugu); chemical balance (Gallenkamp, England); micro – pipettes (Perfect, USA); microscope slides; capillary tube; Refrigerator (Haier thermocool, England), microscope (XSZ – 107BN, India); and counting chamber (MC Qiujing, China).

Animal samples used

Adult male rats Wistar strain (80 – 120 g) and adult Swiss albino mice (20 – 30 g) of both sexes obtained from the animal holding unit of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka were used in the study. The animals were housed under standard conditions ($25 \pm 2\ ^0$C and 12 – hr light/dark cycle). The rats and mice were fed two times in a day with standard pellets (Grand Cereals Ltd, Enugu Nigeria) and had unrestricted access to clean drinking water. The guide for the care and use of laboratory animals procedures were followed in this study (Indian Council of Medical research, 2001).

Plant Materials

The leaves and root of *Robinia pseudoacacia* used for this study were collected from Udi town in Udi Local government area of Enugu State, Nigeria. The leaves and root were identified and authenticated by a taxonomist.

Extraction of Plant Materials

The fresh roots and leaves of *Robinia pseudoacacia* were harvested and dried to constant weight at room temperature (29^0C - 35^0C) for three weeks. The dried roots and leaves were pulverized into coarse form with a Crestor high speed milling machine. The methanol extraction of pulverized roots and leaves of *Robinia pseudoacacia* was carried out. A weighted quantity of the ground roots and that of leaves of *Robinia pseudoacacia* was macerated in 1.5 litres of methanol for 72 hrs, after which it was filtered with a mesh of 0.15nm, followed by filtration with Whatman filter paper. The filtrate was concentrated using Rotary evaporator at regulated temperature to separate the solvent from the extracts.

Fractionation of the Extracts

The dried methanol extracts (40 g) were fractionated by column chromatography using methanol and ethyl-acetate as solvents. Silica gel (250 g) was homogenized with the dried methanol extract using vacuum pump and then parked inside a column for column chromatography. The column was eluted in succession with 1.3 litres of ethyl acetate, and 1.3 litres methanol. The extract was concentrated and evaporated to dryness using rotary evaporator at an optimum temperature to avoid inactivation of the active ingredients. The samples were stored in the refrigerator for subsequent studies.

Proximate Analysis

The Proximate analysis was determined according to the methods of AOAC (2000).

Ash Content

Ash is the inorganic residue obtained by burning off the organic matter of sample at 400-600^{0}C in muffle furnace for 4 hrs. Two grammes (2 g) of the sample were weighed into a platinum crucible. The crucible was placed into muffle furnace at 400-600 ^{0}C for 4 hrs, until whitish-grey ash was obtained. The crucible was then placed in the desiccator and weighed.

$$\% \text{ Ash} = \frac{\text{Wt. of Ash}}{\text{Wt. of the sample}} \times 100$$

Moisture Content

Moisture content is based on an indirect distillation method (Evaporation of moisture). The amount of moisture in the sample is the loss in weight after drying in the oven at 105^{0}C until a constant weight is recorded.

Procedure

Sample (2.0 g) was weighed and dried in the oven at 105 ^{0}C to a constant weight. The dishes and sample were cooled and weighed. The moisture content was then calculated from the equation below.

$$\% \text{ Moisture} = \frac{[\text{Wt. of sample + dish before drying}] - [\text{wt. of sample + dish after drying}]}{\text{Wt. of sample taken}} \times 100$$

Fat Determination

Principle: The sample was continuously extracted with petroleum ether, using an extraction apparatus. After extraction, the petroleum ether was evaporated to dryness and the residue designated the petroleum ether extract. This was referred to as the fat portion of the sample.

Procedure

An anhydrous diethyl ether (petroleum ether) (150 ml) of boiling point of 40 - 60 ^{0}C was placed in the flask. Sample (2 g) was weighed into a thimble and the thimble was plugged with cotton wool. The thimble with content was placed into the extractor; the ether in the 2 flasks was then heated. As the ether vapour reached the condenser through the side arm of the extractor, it condensed to liquid form and drop back into the sample in the thimble, the ether soluble substances were dissolved and were carried into solution through the siphon tube back into the flask. The extraction continued for 4 hrs. The thimble was removed and most of the solvent was distilled from the flask into the extractor. The flask was then disconnected and placed in an oven at 65 ^{0}C for 4 hrs, cooled in a desiccator and weighed.

$$\% \text{ Fat} = \frac{[\text{Wt. of flask + Extract}] - [\text{tare wt. of flask}]}{\text{Wt. of sample}} \times 100$$

Crude Fibre

Principle: This is insoluble and combustible organic residue which remains after the sample has been heated under prescribed condition. The most common conditions are consecutive treatments with light petroleum ether in boiling dilute sulphuric acid, boiling dilute sodium hydroxide, dilute hydrochloric acid, alcohol and ether. This treatment provides a crude fibre consisting largely of the cellulose content together with a proportion of the lignin and hemicelluloses content of the sample.

Procedure:
The defatted sample (2.0 g) was transferred into 500 ml flask and 200 ml of pre-heated 1.25 % H_2SO_4 was added and the solution was gently boiled for 30 min, maintaining constant volume of acid by the addition of hot water. Residue was washed three times with hot water and returned to the flask. Then 200 ml of pre-heated 1.25 % Na_2OH was added and boiled for another 30 min. This was filtered under suction and washed thoroughly with hot water and twice with ethanol. The residue was dried at 65 ^{0}C for about 24 hrs and weighed. The residue was transferred into a crucible and placed in muffle furnace (400-600 ^{0}C) and ashed for 4 hrs. It was then cooled in a desiccator and weighed.

$$\% \text{ Crude fibre} = \frac{[\text{Dried wt. of residue before ashing}] - [\text{wt. of residue after ashing}]}{\text{Wt.of sample}} \times 100$$

Protein Determination
Principle: The crude protein content was determined using the micro kjedahl method. The method is based on the wet combustion of the sample by heating with concentrated sulphuric acid in the presence of metallic and other catalysts to effect the reduction of organic nitrogen in the sample to ammonia, which is retained in solution as ammonium sulphate. The digest having been made alkaline, is distilled to remove ammonia which is trapped and titrated.

Procedure: This involves three major steps:
Digestion: Sample (2 g) was weighed out and carefully transferred into kjeldahl digestion flask. After that, 25 ml of concentrated sulphuric acid, 0.5 g of copper sulphate, 5 g of sodium sulphate and a speck of selenium tablet were added. The flask was put inside a fume cupboard and was heated slowly at first to prevent undue frothing. Digestion continued for 45 minutes until the digest became clear pale green. This was left until completely cooled. Distilled water (100 ml) was added. The mother liquor was poured into a beaker (250 ml). The digestion flask was rinsed 2-3 times and the rinsing added to the bulk in the beaker.

Distillation: Markham distillation apparatus was used for distillation. The distillation apparatus was heated and 10 ml of the digest was added into the apparatus via a funnel and allowed to boil. Sodium hydroxide (10 ml) of 50 % was added from the measuring cylinder so that ammonia was not lost. This was distilled into 50 ml of 2 % boric acid containing screened methyl red indicator.

Titration: The alkaline ammonium borate formed was then titrated directly with 0. 01N HCl. The titre value which is the volume of acid used was recorded.

$$\% \text{ protein} = \frac{\text{Titre x 0.01n HCl x 14.01 (At.utN) x 100 x 50}}{\text{1000 x 0.5g x 10}} \times 100$$

Qualitative Phytochemical Analyses of Roots and Leaves of *Robinia pseudoacacia:*

Tannins
Sample (0.5 g) was stirred with 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. The occurrence of a blue-black, green or blue-green precipitate indicated the presence of tannins (Trease and Evans, 2002).

Flavonoids
A few quantity of the sample was dissolved in water and filtered. To this 2 ml of the filtrate, few drops of 10% ferric chloride solution were added to produce a green-blue or violet colouration. A change in colour from green-blue or violet on addition of dilute ferric chloride was an indication of the presence of flavonoids (Trease and Evans, 2002).

Alkaloids
A few quantity of the sample was stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. From the filtrate, 1 ml was taken individually into 2 test tubes. To the first portion, few drops of Dragendorff's reagent were added; occurrence of orange-red precipitate was taken as positive. To the second, 1 ml

of Mayer's reagent was added and appearance of buff-coloured precipitate was an indication for the presence of alkaloids (Sofowora, 1993).

Saponins

Sample (1 g) was boiled with 5 ml of distilled water and filtered. To the filtrate, 3 ml of distilled water was added and shaken vigorously for 5 minutes. Frothing which persisted on warming was taken as an evidence for the presence of saponins (Trease and Evans, 2002).

Determination of Phenol

A few quantity of the sample was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide was later added to produce a yellow colouration. A change in colour from yellow to colourless on addition of dilute hydrochloric acid was an indication for the presence of phenol (Trease and Evans, 2002).

Determination of Steroids

To 0.2 g of the samples, 2 ml of acetic acid was separately added; the solution was cooled well in ice-bath followed by the addition of conc. H_2SO_4 drop-wise carefully. Colour development from violet to blue or bluish-green indicates the presence of a steroidal ring i.e. a glycone portion of cardiac glycoside (Sofowora, 1993).

Determination of Glycoside

The sample (0.2 g) was dissolved in glacial acetic acid. Later, 2 ml of the filtrate was pipetted into a test tube and 10 ml water was added. This was boiled for 30 minutes and 2 ml of dilute ammonia was added. Then, 0.4 ml Fehling's solution A and B was added and boiled again for 5 minutes and a colour change of brick red shows the presence of glycoside (Trease and Evans, 2002).

Quantitative phytochemical analyses of roots and leaves of *Robinia pseudoacacia*

Determination for Tannin

Tannins content of the root of *Robinia pseudoacacia* was determined by Folin Denis colorimetric method of Nwaokonkwo (2009). Each sample (1 g), was macerated with 20ml of distilled water and filtered. The filterate (5 ml) was pippeted into a test tube. To this added 0.3ml of 0.1N ferric chloride in 0.1N HCl and 0.3ml of 0.0008M potassium ferricyanide were added. This was mixed well. Measurement of absorbance was at 720 nm.

$$\text{Tannin} = \frac{\text{Abs}-\text{blk}}{\text{Slope}} \text{ x } \frac{\text{Volume of solvent}}{\text{Wt. of sample}}$$

Determination of Flavonoid

Flavonoid was determined according to the method of El-Olemyl (1994). A quantity, 1 g of the extract was macerated with 20 ml ethyl-acetate and filtrated. The filterate (5 ml) was pipetted inside the test tube. The dilute ammonia (5 ml) was added and we collect the upper layer. Measurement of absorbance was at 490 nm.

$$\text{Flavonoid} = \frac{\text{Abs}-\text{blk}}{\text{Slope}} \text{ x } \frac{\text{Volume of solvent}}{\text{Wt. of sample}}$$

Determination of Alkaloid

Alkaloid was determined according to the method of El-Olemyl (1994). A portion (1 g) of the extract was macerated with 20 ml of ethanol 20 % sulphuric acid (1:1) and filtrated. Each sample (1 ml) of the filtrate and 5 ml of 60 % H_2SO_4, mix and allowed to stand for 3 hrs. Measurement of absorbance was at 490 nm.

$$\text{Alkaloid} = \frac{\text{Abs}-\text{blk}}{\text{Slope}} \text{ x } \frac{\text{Volume of solvent}}{\text{Wt. of sample}}$$

Determination of Saponin

Saponin was determined according to the method of El-Olemyl (1994). A quantity, 1 g of the extract was macerated with 20 ml of petroleum ether and decanted into a beaker, wash again with 10 ml of petroleum ether, combine the filtrate and evaporate to dryness. The residue was dissolved with 6 ml of ethanol and transferred 2 ml of it into a test tube and added 2 ml of chromogen solution. It was allowed to stand for 30 minutes. Measurement of absorbance was at 550 nm.

$$\text{Saponin} = \frac{\text{Abs}-\text{blk}}{\text{Slope}} \text{ x } \frac{\text{Volume of solvent}}{\text{Wt. of sample}}$$

Determination of phenol

Phenol was determined according to the method of El-Olemyl (1994). A quantity, 1 g of the extract was macerated with 20 ml of 80% ethanol and filter. Each sample (5 ml) of the filtrate was added inside test tube. The folinciocalteus reagent (0.5 ml) was added and after 30 minutes, 2 ml of 20% sodium carbonate was added. The absorbance was measured at 650 nm.

$$\text{Phenol} = \frac{\text{Abs}-\text{blk}}{\text{Slope}} \text{ x } \frac{\text{Volume of solvent}}{\text{Wt. of sample}}$$

Determination of steroids

This was determined according to the method of El-Olemyl (1994). A quantity, 2 g of the extract was macerated with 20 ml of ethanol and filter. Each sample (2 ml) of filtrate and 2 ml ethanol was pipette inside the test tube. After that, 2 ml of colour reagent was added in sample test tube and blank test tube, and was left to stand for 30 minutes. The absorbance was measured at 550 nm.

$$\text{Steroids} = \frac{\text{Abs}-\text{blk}}{\text{Slope}} \text{ x } \frac{\text{Volume of solvent}}{\text{Wt. of sample}}$$

Determination of glycoside

This was determined according to the method of El-Olemyl (1994). A quantity, 1 g of the extract was macerated with 20 ml of distilled water and filter. Add 2.5 ml of 15% lead acetate and we filter and add 2.5 ml of chloroform, Shake vigorously. The lower layer was collected and evaporated to dryness. The residue was dissolved with 3 ml of glacial acetic acid and 0.1 ml of 5% ferric chloride was added. The concentrated H_2SO_4 (0.25 ml) was added and kept in the dark for 2 hours. The absorbance was measured at 530 nm.

$$\text{Glycoside} = \frac{\text{Abs}-\text{blk}}{\text{Slope}} \text{ x } \frac{\text{Volume of solvent}}{\text{Wt. of sample}}$$

Acute Toxicity Studies (LD_{50})

The acute toxicity studies of the crude methanol extract, ethyl acetate and methanol fractions were estimated in mice using the method of Lorke (1983). The tests involved two phases. The first phase was determination of the toxic range. The mice were placed in three groups of 5 mice each and were given 10, 100 and 1000 mg/kg body weight of the extract solubilised in 2.5%, v/v propylene glycol in water. The treated mice were observed for 24 hrs for number of deaths. The death pattern in the first phase determined the doses used for the second phase. In the second phase, the mice received 1600, 2900 and 5000 mg/kg body weight of the extract. The treated animals were observed for 24 hrs for lethality or signs of acute intoxication.

Chronic Toxicity Test

Repeat-dose oral toxicity study was carried out according to the OECD guideline 407. The animals were divided into three groups of 5 animals each. The mice received extract doses of 10, 100 and 1000 mg/kg body weight of extract solubilised in 2.5%, v/v propylene glycol in water. The extracts were administered daily for 90 days respectively and observed at least twice daily for morbidity and mortality. In the second phase, the mice received 1600, 2900 and 5000 mg/kg body weight of the extract. The extracts were administered daily for 90 days respectively and observed for lethality or sign of intoxication.

Experimental Design: The study was carried out in stages as follows:

Animal Grouping

Forty five male albino rats divided into nine groups of five rats each were used for this study. They represented groups receiving different doses of crude methanol extract, methanol and ethyl acetate fractions of the plant sample. Group 1 is the normal control (Not induced and untreated), Group 2 is the positive control (Induced and Untreated), and Group 3 was induced and treated with standard drug, dutesteride (Avodart) and served as standard control. Groups 4 and 5 were induced with BPH and treated with 200 mg/kg and 300 mg/kg body weight for crude extract respectively. Groups 6 and 7 were induced with BPH and treated with 200 mg/kg and 300 mg/kg body weight of methanol fraction respectively. Groups 8 and 9 were induced with BPH and treated with 200 mg/kg and 300 mg/kg body weight of ethyl acetate fraction respectively.

Proximate Composition of Black Locust *(Robinia pseudoacacia)*

Sample	Protein (%)	Ash (%)	Moisture (%)	Fats (%)	Fibre (%)
Leaf 1	14.95	2.69	28.63	22.17	0.25
Leaf 2	14.81	2.72	27.92	22.09	0.31
Root 1	21.03	2.01	19.71	9.88	ND
Root 2	20.78	1.87	19.90	10.23	ND

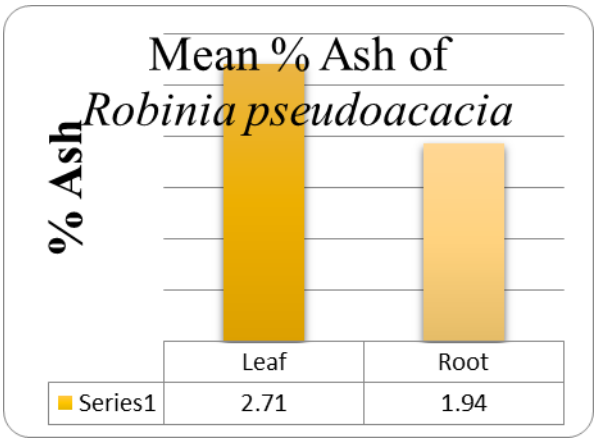
ND = Not Detected.

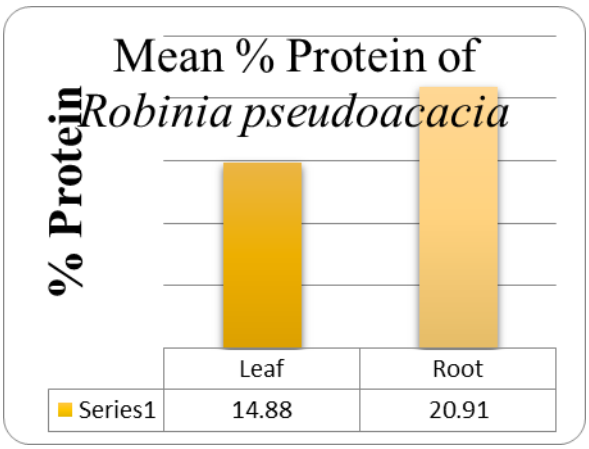
Mean Proximate Composition of Black Locust *(Robinia pseudoacacia)*

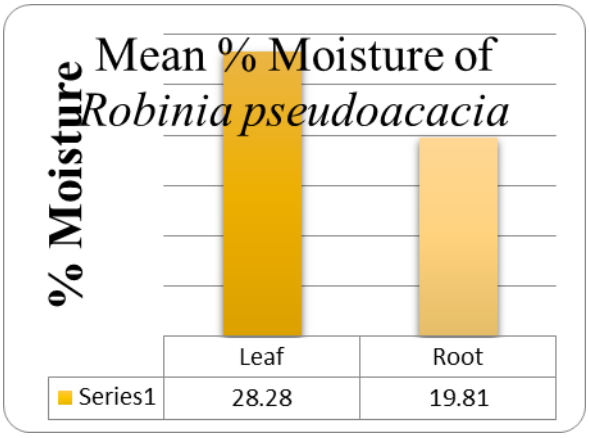
Sample	Protein (%)	Ash (%)	Moisture (%)	Fats (%)	Fibre (%)
Leaf	14.88 ±0.09	2.71± 0.02	28.28 ±0.51	22.13 ±0.05	0.28± 0.04

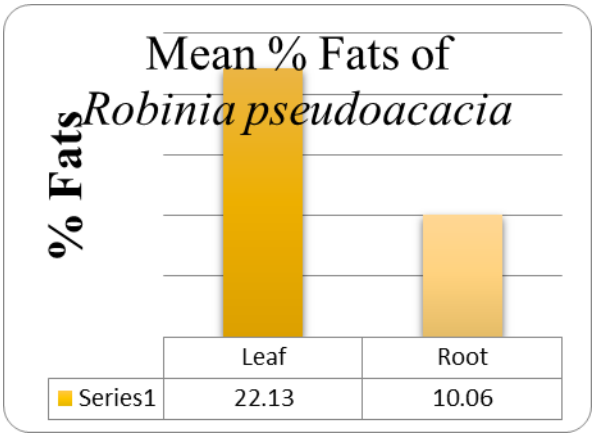
Root	20.91 ±0.18	1.94± 0.09	19.81 ±0.13	10.06 ±0.25	ND

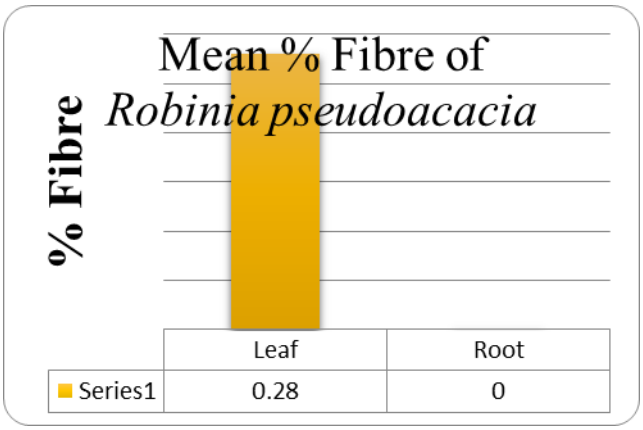
ND = Not Detected.









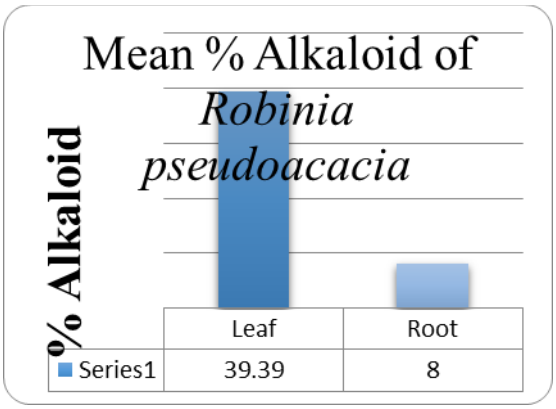


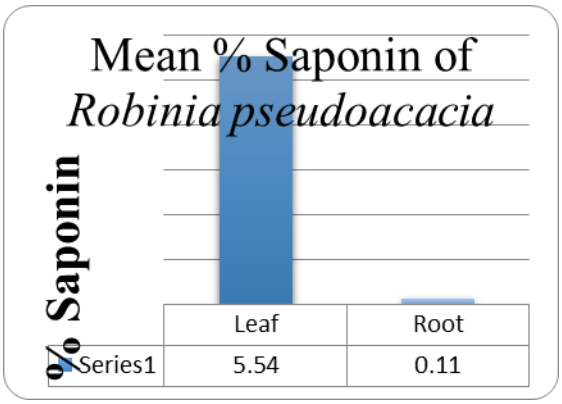
Phytochemical Composition of Black Locust *(Robinia Pseudoacacia)*

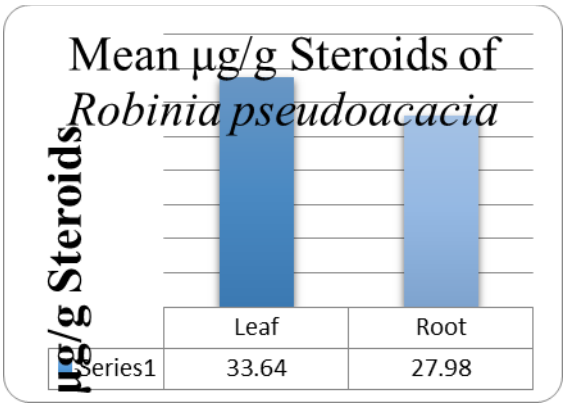
Sample	Saponin (%)	Flavonoid (mg/g)	Steroids (µg/g)	Alkaloid (%)	Tannin (%)	Glycoside (mg/100g)
Leaf 1	5.63	5.787	33.79	40.00	0.288	64.12
Leaf 2	5.44	5.665	33.48	38.78	0.303	66.09
Root 1	0.10	1.856	27.73	7.56	0.219	27.36
Root 2	0.12	1.888	28.22	8.43	0.222	27.71

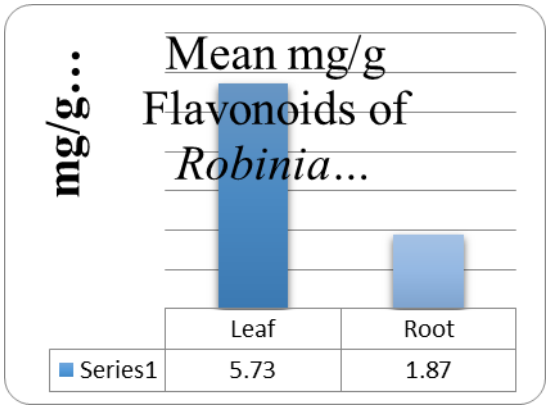
Mean Phytochemical Composition of Black Locust *(Robinia pseudoacacia)*

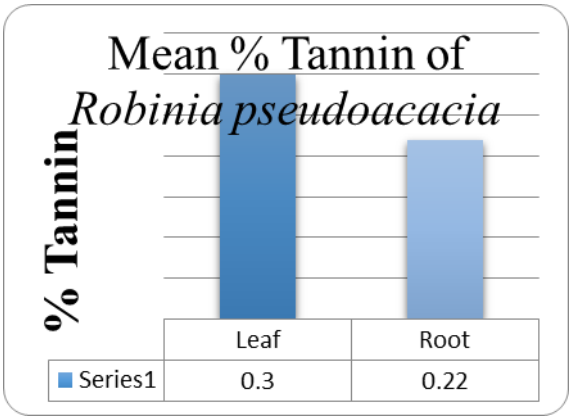
Sample	Saponin (%)	Flavonoids (mg/g)	Steroids (µg/g)	Alkaloid (%)	Tannin (%)	Glycoside (mg/100g)
Leaf	5.54 ±0.13	5.73 ±0.09	33.64 ±0.22	39.39 ±0.86	0.30 ±0.01	65.11±1.39
Root	0.11 ±0.16	1.87 ±0.02	27.98 ±0.35	8.00 ±0.62	0.22 ±0.002	27.54±0.23

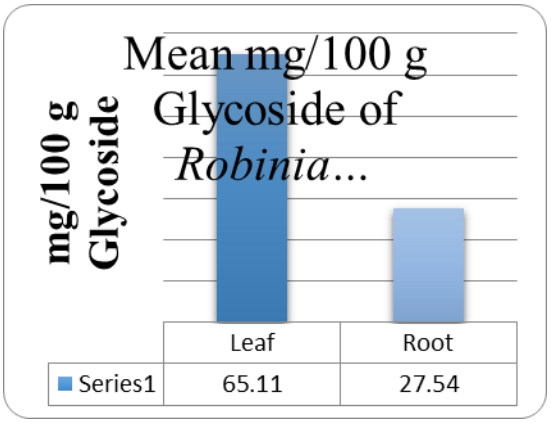












Functional Groups Composition of Black Locust *(Robinia pseudoacacia)*

Sample	Alkene Group	Phenolic Group	Amino Group	Carboxylic Group	Alcoholic Group	Aldehyde Group
Leaf	+ +	+ +	+ +	+ + +	-	+ +
Root	+ +	+	+	+ +	+	-

Keys:
+ = present; + + = moderately present; + + + = excessively present; - = absent.

Acute Toxicity Test on Leaf Extract
Stage 1
10 mg/kg concentration was given to the first group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: A1=45, A2=46, A3=40.
100 mg/kg concentration was given to the second group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: B1=42, B2=40, B3=44.
1000 mg/kg concentration was given to the third group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: C1=39, C2=38, C3=39.
Observation
After the 24 hour period, none of the mice died and there were no signs of intoxication.

Stage 2
1600 mg/kg concentration was given to the first group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: A1=41, A2=40, A3=44.

2900 mg/kg concentration was given to the second group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: B1=40, B2=38, B3=36.

5000 mg/kg concentration was given to the third group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: C1=35, C2=39, C3=40.

Observation
After the second stage also, none of the animals died and there were no signs of intoxication in all the mice.
Acute Toxicity Test on Root Extract

Stage 1
10 mg/kg concentration was given to the first group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: A1=29, A2=34, A3=28.

100 mg/kg concentration was given to the second group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: B1=40 , B2=38, B3=39.
1000 mg/kg concentration was given to the third group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: C1=32, C2=33, C3=30.

Stage 2
1600 mg/kg concentration was given to the first group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: A1=40, A2=42, A3=44.

2900 mg/kg concentration was given to the second group for 24 hours. The group's test subjects (mice) serial numbers and weights are as follows: B1=40 , B2=39, B3=38.

5000 mg/kg concentration was given to the third group for 24 hours. The group's test subjects (mice) serial

numbers and weights are as follows: C1=35, C2=36, C3=35.

Observation

For both Stage 1 and Stage 2, none of the mice died and there were no signs of toxicity.

Chronic Toxicity Test for Both Leaf and Root Extracts

S/N	Body weights (mg/kg)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
A1	152	24	38	52
A2	150	22	36	50
A3	148	28	35	56
B1	139	26	35	54
B2	140	24	36	49
B3	142	20	34	52
C1	144	23	33	50
C2	140	25	35	53
C3	150	26	36	52
D1	146	28	34	56
D2	152	22	35	50
D3	143	26	39	54
E1	149	25	35	52
E2	158	22	32	53
E3	152	24	33	51

Keys:

Group A received 1000 mg of leaf extract for 14 days

Group B received 3000 mg of leaf extract for 14 days

Group C received 1000mg of root extract for 14 days

Group D received 3000mg of root extract for 14 days

Group E received Normal saline for 14 days

III. DISCUSSION

The Elucidation and physicochemical analysis of bioactive composition of leaves and roots methanol extract of *Robinia pseudoacacia* (black locust) is carried out successfully. All chemicals and reagents used in this study were of analytical grade. Mean Proximate Composition for both the leaves and the root gives (%)Protein14.88±0.09, 20.91±0.18; (%)Ash 2.71±0.02, 1.94±0.09; (%)Moisture 28.28±0.51, 19.81±0.13; (%)Fats22.13±0.05, 10.06±0.25; and (%)Fibre 0.28±0.04, ND. Mean Phytochemical Composition for both the leaves and the root respectively gives (%)Saponin 5.54±0.13, 0.11±0.16; (mg/g)Flavonoids 5.73±0.09, 1.87±0.02; (µg/g)Steroids 33.64±0.22, 27.98±0.35; (%)Alkaloid 39.39±0.86, 8.00±0.62; (%)Tannin 0.30±0.01, 0.22±0.002; and (mg/100 g)Glycoside 65.11±1.39, 27.54±0.23. Acute toxicity test was carried out on both leaves and roots extract of the plant. For both Stage 1 and Stage 2 of the test, none of the animals died and there were no signs of intoxication in all the mice. Chronic toxicity test was also done for both leaf and root extracts and the results showed no sign of intoxication in the tested mice. Functional groups composition of the *Robinia pseudoacacia* was also determined. Both the leaves and roots moderately contain alkene groups. The leaves have more phenolic and amino groups than that of the roots. Carboxylic acid is contained in both the leaves and the roots but it is more pronounced in the leaves. There is no alcoholic group in the leaves but it is present in the root at a very low concentration. Aldehyde group is present in the leaves but absent in the roots.

CONCLUSION

This research revealed that the Black Locust (*Robinia pseudoacacia)* trees grown in the South Eastern part of Nigeria does not show sign of toxicity to animals. This means that both the leaves and roots of the tested black locust contain no toxalbumins which are the toxic proteins responsible for the tree's toxic nature.

ACKNOWLEDGEMENT

The authors are grateful to Tertiary Education Trust Fund (TETFund) for providing the Fund for the Research work through the Institution Based Research

(IBR) Programme. The authors also appreciate their parent Institution, Institute of Management and Technology (IMT), Enugu Nigeria for the enabling environment during the research period.

REFERENCES

[1] Demene, J.M. and Merzeau, D. (2007). Black Locust. History and Biological Characteristics. *Foreˆt enterprise,* 177:10–12.

[2] Diggs, G.M. Jr.; Lipscomb, B.L and O'Kennon, R.J. (1999). Shinners and Mahler's illustrated Flora of North Central Texas. *Botanical Research Institute of Texas*: Pp: 162.

[3] El-Olemyl M.M, FJ.Al-muhtadi and A.A. Afifi (1994). Experimental Phytochemistry: A laboratory manual. King saud University Press, Saudi Arabia, Pp: 3-19

[4] Huntley, J. C. (1990). *Robinia pseudoacacia* (Black locust): An Agricultural Handbook. Volume 2. Washington, DC. U.S. Department of Agriculture, Forest Service. Pp: 755-761.

[5] Ivanschi, T.; Costea, A.; Bıˆrla˘nescu, E.; Marcoiu, A. and Nonute, I. (1969). Research on the Selection of Sites Suitable for Black Locust Culture. In *Research on Black Locust Culture*.

[6] Laird, F.J. (1995). Trees of the Northern United States and Canada. Ames, IA: Blackwell Publishing. Pp: 502.

[7] Lavnyy, V. and Savchyn, V. (2016). Ukraine. In: Hasenauer H, Gazda A, Konnert M, Mohren G, Po¨tzelsberger E, Spiecker H, van Loo M (eds) Non-native Tree Species for European Forests: Experiences, *Risks and Opportunities.* Joint volume. Vienna, University of Natural Resources and Life Sciences. Pp 353–360.

[8] Lorke D (1983). A New Approach to Practical Acute Toxicity Testing. *Arch Toxicol. 54:275-87*

[9] Mirancea, I. (2002). Research on *In-vitro* Selection of Some Forest Trees Genotypes Resistant to Abiotic Stress. *Analele ICAS,* 45(1):31–38.

[10] Sofowora, A. (1993). Medicinal plants and medicine in Africa. John Wiley Spectrum, Ibadan Nigeria. Pp: 81-85

[11] Trease, G.E and Evans, W.C (2002). Pharmacognosy, 13th BelliereTindall London. Pp: 386