



RESEARCH ARTICLE

COMPARATIVE ANALYSIS OF THE PHYTOCHEMICAL COMPOSITIONS OF LEAF, STEM-BARK AND ROOT OF *AZADIRACHTA INDICA* (NEEM)

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Abstract

Objective: Plant chemicals abound in different parts of plants, in different compositions. Thus, the comparative screening of the leaf, stem-bark and root of *Azadirachta indica* becomes imperative. The presence of nine phytochemicals which include; alkaloids, flavonoids, saponins, glycosides, phenols, steroids, tannins, reducing sugars and anthraquinones, and the quantity of alkaloids, flavonoids, saponins, tannins and glycoside in the leaf, stem-bark and root of *Azadirachta indica* were investigated.

Methods: The plant parts were collected from a plantation grown in Okpotegu Echara, Ikwo Local Government Area of Ebonyi State, Nigeria. Plant samples were air dried at room temperature, ground into uniform powder, sieved, bottled and labeled, ready for physico – chemical analysis.

Results: Alkaloids were not detectable in leaf, stem-bark and root samples of aqueous extract. Glycosides were not detectable in leaf sample of ethanol and aqueous extracts. Quantitatively, the phytochemical compositions of each part showed higher concentrations of Alkaloids in the leaf, stem-bark and root (11.63%, 4.93% and 3.79%), compared to flavonoids (2.19%, 2.72% and 0.92%), saponins (0.70%, 1.12% and 0.44%), tannins (0.33 mg/100, 0.50 mg/100 and 0.17 mg/100) and glycosides (0.23%, 0.27% and 0.19%), respectively.

Conclusion: Obviously, except for the higher percentage (11.63%) of alkaloids in the leaf, the phytochemicals in the stem-bark are higher as shown by the results, which could support the reason that the bark is preferably chewed commonly together with the stem as chew stick for its germicidal and antifungal action.

Keywords: Analysis, composition, concentration, extracts, plant chemicals, screening.

INTRODUCTION

Plants are nature's gift by God to man for his beneficial herbal exploits in divers applications including herbal traditional medicine, antimicrobial, antifungal, biogas production, biofertilizers and antiseptic^{1,2,3}. Plant chemicals are referred to as phytochemicals. Several research works have identified thousands of these different plant chemicals, which were found in vegetables, fruits, beans, whole grains, nuts and seeds⁴. Phytochemicals are chemical compounds produced by plants, generally to help them thrive or fight against predators or pathogens. The name comes from the Greek word phyto, which means plant. Some phytochemicals have been used as traditional medicine, as poison and as nutrients⁵. Phytochemicals which are

naturally contained in plants with known beneficial roles in the body have been classified as essential nutrients in diet, for the body's normal physiological functions⁵. However, Iwasaki⁶ Bjeldanes and Shibamoto⁷, have reported the phytotoxicity and antinutrients value of some phytochemicals to humans, as aristolochic acid is carcinogenic at low doses and some interfere with nutrients absorption. *Azadirachta indica* species is a medium to large size evergreen tree of the tropical and sub-tropical regions of the world and is native of India. It is commonly known as Neem tree. In Nigeria, it is called Atuyabasi in Igbo, Odogoyalo in Idoma, Maina in Hausa and Dongoyaro in Yoruba. It belongs to the family of *Meliaceae* and has been used as a source of drugs in many traditional African societies like Nigeria⁸. Researchers have

reported the various uses of Neem seeds, fruits, oils, leaves, bark and root as general antiseptic, antimicrobial, and treatment of disorders (such as urinary, diarrhea, fever, bronchitis, skin disease, septic sores, hypertension, infected burns and inflammatory disease)⁹. Screening of phytochemicals involves the extraction, screening and identification of bioactive substances (plant chemicals) in plants. Some of the phytochemicals were found in plants include; tannins, flavonoids, alkaloids, phenols, glycosides, carotenoids, antioxidants, steroids and saponins¹⁰. These bioactive ingredients in *A. Indica* are present in different detectable concentrations and compositions¹¹. Phytochemicals of many plants have been assayed right from onset and in pest control, bioactive plant extracts, such as rotenone, pyrethrum and nicotine have been used¹². Interestingly, many researchers in Biochemistry, Pharmacology and Botany have their interest increased in phytochemical screening of plants for the presence of phytochemicals, for the development of medicine, pesticides and germicide functions. Feng and Isman¹³ doubtlessly appreciate the difficult and expensive process involved in the screening, isolation and identification of plants' secondary metabolites produced in large quantity to be commercialized. He pointed out that at least nine neem limonoids have demonstrated an ability to block insect growth, affecting a range of species that includes some of the most deadly pests of agriculture and human health¹³. In a phytochemical study carried out by Harry-Asobara and Eno-Obong¹², Neem leaf in comparison with the other parts of the plant gave greater percentage of alkaloid (1.38), flavonoid (0.44), and saponin (0.72). Neem seed contained greater percentage of HCN (13.04) and phytate (0.32) than other parts of the plants while greater percentage of tannin was observed in Neem bark (0.26) followed very closely by Neem Seed (0.24), while Neem leaf and Neem bark contained the same phenolic percentage (0.18).

MATERIALS AND METHODS

Sample Collection and Preparation

Fresh undamaged mature leaves were collected from several parts of the inner most canopies of the Neem tree as well as stem-barks and roots. These samples were obtained from Okpotegu Echara village in Ikwo Local Government Area of Ebonyi State. The preparation of samples collected from the field was done according to methods described by Edeoga *et al.*,⁸ The leaf, stem-barks and roots collected were air dried at room temperature. The ground samples were then sieved, obtain powdered material, bottled and labeled and were ready for physicochemical analysis.

Phytochemical screening

This was performed in aqueous and ethanol extracts of *A. indica* leaf, stem-bark and root using standard procedure to identify the constituents as described by Harborne¹⁴; Boham and Abyazan¹⁵; Obadonic and Ochuko¹⁶; Van Burden and Robinson¹⁷; Singh, *et al.*,¹⁸; Trease and Evans¹⁹.

RESULTS

The phytochemical qualitative screening (Table 1) in ethanol and aqueous leaf extracts respectively showed that, alkaloids were very deeply present (+++) and deeply present (++); flavonoids were very deeply present(+++) and present (+); saponins in frothing form were present (+) in both extracts, saponins in emulsion form were very deeply present (+++) and deeply present; cyanogenic glycosides were very deeply present (+++) and deeply present (++) while cardiac glycosides were not detectable (ND) and present (+); phenols were very deeply present (+++) in both extracts; steroids were not detectable (ND) in both extracts; tannins were very present (++) and present (+); and reducing sugars and anthraquinones were not detectable (ND) in both extracts.

Meanwhile, the quantitative analysis of leaf of *A. indica* (Table 4) reveals the phytochemical distribution in a decreasing order: Alkaloids (11.63%), flavonoids (2.19%), saponins (0.70%), tannins (0.33 mg/100) and glycoside (0.23%). Table 2 shows the qualitative screening of stem-bark in ethanol and aqueous extracts respectively. Alkaloids were deeply present (++) and not detectable (ND); flavonoids were very deeply present (+++) in both extracts; frothing saponins were deeply present (++) and very deeply present (+++) while emulsion saponins were very deeply present (+++) and deeply present (++); cyanogenic glycosides and cardiac glycosides were very deeply present (+++) in both extracts; phenols were very deeply present (+++) in both extracts; steroids were deeply present (++) and present (+); tannins were very deeply present (+++) and deeply present (++); reducing sugars were present (+) in ethanol extract but not detectable (ND) in aqueous extracts; while anthraquinones were not detectable (ND) in both extracts. However, Table 4 reveals the phytochemicals quantitative analysis of *A. indica* stem-bark in a decreasing order: alkaloids (4.93%), flavonoids (2.72%), saponins (1.12%), tannins (0.5 mg/100) and glycosides (0.27%). Table 3 shows the qualitative screening of root in ethanol and aqueous extracts respectively. Alkaloids were deeply present (++) and not detectable (ND); flavonoids were present (+) and not detectable (ND); frothing saponins were present (+) and deeply present (++) while emulsion saponins were deeply present (++) and present (+); cyanogenic glycosides were deeply present in both extracts while cardiac glycosides were present (+) and deeply present (++); phenols were very deeply present (+++) and deeply present (++); steroids were not detectable (ND) in both extracts; tannins were present (+) in both extracts, while reducing sugars and anthraquinones were not detectable in both extracts. Following this (Table 4), the quantitative screening of root of *A. indica* reveals a decreasing pattern of the phytochemicals except glycosides (0.19%) which is slightly higher than tannins (0.17%) : alkaloids (3.79%), flavonoids (0.92%), saponins (0.44%), tannins (0.17 mg/100) and glycosides (0.19%).

Table 1: Phytochemical screening of leaf of *A. indica* in ethanol and aqueous extracts.

Phytochemicals	Leaf ethanol extract	Root ethanol extract
Alkaloids	+++	++
Flavonoids	+++	+
Saponins	+	+
(a). Frothing	+++	++
(b). Emulsion		
Glycosides	+++	++
(a). Cyanogenic	ND	+
(b). Cardiac		
Phenols	+++	+++
Steroids	ND	ND
Tannins	++	+
Reducing Sugars	ND	ND
Anthraquinones	ND	ND

Very deeply present- (+++), Deeply present- (++) , Present- (+), Not detectable -(ND)

Table 2: Phytochemical screening of stem–bark of *A. indica* in ethanol and aqueous extracts.

Phytochemicals	Stem-bark ethanol extract	Stem-bark aqueous extract
Alkaloids	++	ND
Flavonoids	+++	+++
Saponins	++	+++
(a). Frothing	+++	++
(b). Emulsion		
Glycosides	+++	+++
(a). Cyanogenic	+++	+++
(b). Cardiac		
Phenols	+++	+++
Steroids	++	+
Tannins	+++	++
Reducing Sugars	+	ND
Anthraquinones	ND	ND

Very deeply present- (+++), Deeply present- (++) , Present- (+), Not detectable -(ND)

DISCUSSION

The qualitative phytochemical analysis of *A. Indica* in this study revealed the presence of alkaloids, flavonoids, saponins, tannins, phenols and glycosides in all plant parts studied but to varying intensities while steroids, reducing sugars and anthraquinones were not detectable in leaf and root, anthraquinones were not detectable in all plant parts investigated as shown in Tables 1, 2 and 3. Mallikharjunah *et al.*,²⁰ reported the usefulness of preliminary qualitative test in the detection of bioactive principle that may subsequently result in drug discovery and development.

Anthraquinones were not present in the leaf, stem-bark and root of *A. indica*, irrespective of the extract used; this was also supported by study of Muhammed *et al.*,²¹ on *Acacia nilotica* (Thorn mimosa). Alkaloids were not detected in a study by Aiyelaagbe *et al.*,²² on leaves of *Magnifera indica* but were detected in the leaf of this present study. In a phytochemical study on the seeds of *Artocarpus communis*, *Artocarpus heterophyllus*, *Calophyllum inophyllum*, *Garcinia kola*, *Garcinia mangostana*, *Pentaclethra macrophylla* and *Treulia africana* plants, it was revealed that all the plant specimens were found to contain flavonoids and reducing compounds but none of them contain

phlobatanin, cardiac glycoside, combined anthraquinone, free anthra-quinone, carotenoid and steroids²³, but it is on the contrary to this finding. The qualitative phytochemical screening of *A. Indica* on leaf, stem-bark and root, in this investigation revealed that alkaloids, tannins, saponins, phenols, flavonoids and glycosides abound in a substantial quality that confirms the relatedness of the work carried out by Blessing *et al.*,²⁴ on *Jatropha* species showing these phytochemicals' potentials in drug industry. *A. indica* contains several active ingredients which act in different ways under different circumstances. Feng *et al.*,²⁵ reported that at least nine neem limonoids have demonstrated an ability to block insect growth, affecting a range of species that includes some of the most deadly pests of agriculture and human health. The absence of steroids in leaf and root, and presence in stem-bark extracts of the neem plant in this study could explain why the stem-bark is often used (chewed raw or boiled) as pharmacotherapy for production of sex hormone²⁶. High concentrations of plants' secondary metabolites have been reported to be responsible for many beneficial purposes to include biofertilizers and biogas production and also related to their rodenticidal and pesticidal properties as investigated in previous studies^{1,27} on *Jatropha curcas* seeds to a variety of

insect pests. This is concurrent with this study (Table 4), were the compositions of alkaloids, flavonoids, saponins, tannins and glycosides are distributed in a manner such that alkaloids were higher (11.63%, 4.93% and 3.79%) in leaf, stem-bark and root respectively. However, the concentrations of these

secondary metabolites were seen to progressively increase in stem-bark than in leaf and root. It has been reported that flavonoids are one of the most popular secondary metabolites possessing a variety of biological activities at nontoxic concentrations²⁸.

Table 3: Phytochemical screening of root of *A. indica* in ethanol and aqueous extracts.

Phytochemicals	Root ethanol extract	Root aqueous extract
Alkaloids	++	ND
Flavonoids	+	ND
Saponins	+	++
(a). Frothing		
(b). Emulsion	++	+
Glycosides	++	++
(a). Cyanogenic		
(b). Cardiac	+	++
Phenols	+++	++
Steroids	ND	ND
Tannins	+	+
Reducing	ND	ND
Sugars		
Anthraquinones	ND	ND

ND = Not detected; + = present; ++ = deeply present and +++ = very deeply present.

Table 4: Quantitative phytochemical screening of *A. indica*.

S.N.	Phytochemicals	Leaf	Steam-bark	Root
1	Alkaloids	11.63%	4.93%	3.79%
2	Flavonoids	2.19%	2.72%	0.92%
3	Saponins	0.70%	1.12%	0.44%
4	Tannins	0.33 mg/100	0.50mg/100	0.17mg/100
5	Glycosides	0.23%	0.27%	0.19%

The secondary metabolites *A. indica* investigated in this study, together with those of other plants have been severally reported to show curative activity against diverse pathogens, used traditionally as analgesic, antimicrobial and soothing herbs^{29,30,31}, with dietary flavonoids been known to partake in cancer prevention³². Saponins and tannins have been reported by Addae-Mensah³³ for their medicinal importance as part of the component for traditional medicine (herbal) preparations for the management of various common ailments. The presence and quantity of saponins, flavonoids and phenols in the leaf, stem-bark and root of Neem (*A. indica*) in this study could be that they contain antioxidants, anticancer and anti-inflammatory activities, as reported by Oskoueian *et al.*,³⁴ in the root and latex extracts of *Jatropha curcas*.

CONCLUSIONS

The qualitative and quantitative screening of the investigated phytochemicals in this work, using *A. indica* (leaf, stem-bark and root) revealed that these secondary metabolites in plants occur in different qualities and quantities. Their usefulness to pharmaceutical and other chemical industries for the production of drugs for malaria, hypertension, cancer, antidotes for many poisons, birds and insects repellent and treatment of skin infection, have been more in the traditional or herbal form. More technologies should be

developed for the isolation of these secondary metabolites for their pharmaceutical applications.

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AUTHOR'S CONTRIBUTION

Nwali ON: writing original draft, methodology, investigation. **Idoko A:** formal analysis, data curation, conceptualization. **Okolie JE:** writing, review and editing. **Ezeh E:** methodology, formal analysis. **Ugwudike PO:** data curation, conceptualization. **Ezenwali MO:** writing, review, and editing. **Odo IA:** methodology, data curation. **Ani PN:** writing, review, and editing. **Okolie SO:** writing, review and editing, data curation. Every author gave their approval to the manuscript's final draft.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

Authors have declared that no conflict of interest is associated with this work.

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