Comparative Analysis of the Phytochemical Compositions of Leaf, Stem-Bark and Root of *Azadirachta indica (*Neem)

Abstract

Plant chemical substances abound in different parts of many 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, using standard methods. The plant parts were collected from a plantation grown in Okpotegu Echara, Ikwo Local Government Area of Ebonyi State, Nigeria. After which were air dried at room temperature, ground into uniform powder, sieved, bottled and labeled, ready for physico – chemical analysis. The result of the investigation reveals that anthraquinones were beyond detection limits in all the plant parts tested in both ethanol and aqueous extracts. 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 show 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, 0.50mg/100 and 0.17mg/100) and glycosides (0.23%, 0.27% and 0.19%), respectively. 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: Plant chemicals; Concentration; Screening; Analysis; Extracts; Composition

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, found in vegetables, fruits, beans, whole grains, nuts and seeds [4]. 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 nutrients [5].

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 functions [5]. However, Iwasaki [6] Bjeldanes and Shibamoto [7], have reported the phytotoxicity and antinutrients value of some phytochemicals to humans, which aristolochic acid is carcinogenic at low doses and some interfere with nutrients absorption.

Azadirachta Indica specie 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 Atu yabasi 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 Nigeria [8].

Researchers have reported the various uses of Neem seeds, fruits, oils, leaves, bark and root as general antiseptic, anti-microbial, 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 found in plants include; tannins, flavonoids, alkaloids, phenols, glycosides, carotenoids, antioxidants, steroids and saponins [10]. These bioactive ingredients in *A. Indica* are present in different detectable concentrations and compositions [11]. 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 used [12]. 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 [13]. Feng and Isman, [13] doubtlessly appreciates the difficult and expensive process involved in the screening, isolation and identification of plants' secondary metabolites produced in large quantity to be commercialized and 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 health [13].

In a phytochemical study carried out by Harry-Asobara and Eno-Obong [12], Neem leaf in comparism 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 same phenolic percentage (0.18).

Materials and Method

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. These were then ground into uniform powder using electrical grinding machine. The ground samples were then sieved, obtain powdered material, bottled and labeled and were ready for physico – chemical analysis.

Phytochemical Screening (qualitative and quantitative)

This was performed in aqueous and ethanol extracts of *Azadirachta Indica* leaf, stem-bark and root using standard procedure to identify the constituents as described by Harborne [14]; Boham and Abyazan [15]; Obadonic and Ochuko [16]; Van Burden and Robinson [17]; Singh, et al., [18]; Trease and Evans [19].

Results

Table 1: Phytochemical screening of leaf of *Azadirachta Indica* in Ethanol and Aqueous extracts.

S/N	Phytochemicals	Leaf Ethanol Extract	Root ethanol extract
1	Alkaloids	+ + +	+ +
2	Flavonoids	+ + +	+
3	Saponins (a) frothing	+	+
	(b) Emulsion	+ + +	++
4	Glycosides (a)cyanogenic	+++	+ +
	(b) cardiac	ND	+
5	Phenols	+ + +	+ + +
6	Steroids	ND	ND
7	Tannins	+ +	+
8	Reducing Sugars	ND	ND
9	Anthraquinones	ND	ND

The phytochemical qualitative screening (Table1) 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 Azadirachta Indica (Table 4) reveals the phytochemical distribution in a decreasing order: Alkaloids (11.63%), flavonoids (2.19%), saponins (0.70%), tannins (0.33mg/100) and glycoside (0.23%).

Table 2: Phytochemical screening of stem –bark of *Azadirachta Indica* in Ethanol and Aqueous Extracts

S/N	Phytochemicals	Stem-bark ethanol Extract	Stem-bark aqueous extract
1	Alkaloids	+ +	ND
2	Flavonoids	+ + +	+++
3	Saponins (a) frothing	+ +	+++
	(b) Emulsion	+ + +	+ +
4	Glycosides (a)cyanogenic	+ + +	+ + +
	(b) cardiac	+ + +	+ + +
5	Phenols	+ + +	+ + +
6	Steroids	+ +	+
7	Tannins	+ + +	+ +
8	Reducing Sugars	+	ND
9	Anthraquinones	ND	ND

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 anthraquiones were not detectable (ND) in both extracts. However, Table 4 reveals the phytochemicals quantitative analysis of Azadirachta Indica stem-bark in a decreasing order: Alkaloids (4.93%), flavonoids (2.72%), saponins (1.12%), tannins (0.5mg/100) and glycosides (0.27%).

Table 3: Phytochemical screening of root of *Azadirachta Indica* in Ethanol and aqueous Extracts

S/N	Phytochemicals	Root ethanol extract	Root aqueous extract
1	Alkaloids	+ +	ND
2	Flavonoids	+	ND
3	Saponins (a) frothing	+	++
	(b) Emulsion	++	+
4	Glycosides(a)cyanogenic	+ +	+ +
	(b) cardiac	+	+ +
5	Phenols	+ + +	+ +
6	Steroids	ND	ND
7	Tannins	+	+
8	Reducing Sugars	ND	ND
9	Anthraquinones	ND	ND

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

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 Azadirachta 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 (0.17mg/100) and glycosides (0.19%).

Table 4: Quantitative Phytochemical Screening of *Azadirachta 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%

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 *Azadirachta 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 Treculia 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 anthraquinone, carotenoid and steroids [23], 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 reveals 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 hormone [26].

High concentrations of plants' secondary metabolites have been reported to be responsible for many beneficial purposes to include biofertilizers and biogas production [2] and also related to their rodenticidal and pesticidal properties as investigated by [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 concentrations [28].

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 prevention [32]. Saponins and tannins have been reported by Addae-Mensah [33] 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*.

Conclusion

The qualitative and quantitative screening of the phytochemicals investigated in this work, using *Azadirachta 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 repellant 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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