GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

Check for updates

Phytochemicals of neem plant (*Azadirachta indica*) explains its use in traditional medicine and pest control

Innocent Izuchukwu Ujah [1], Chukwunonso Anthony Nsude [1], Onuabuchi Nnenna Ani [1], Uchenna Blessing Alozieuwa [2], Innocent Oluwaseun Okpako [3] and Amos Ejiofor Okwor [1]

[1] *Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.*
[2] *Department of Biochemistry, Veritas University Abuja, Nigeria.*
[3] *Department of Medical Biochemistry, Faculty of Basic and Medical Sciences, Delta State University, Abraka, Delta State, Nigeria.*

GSC Biological and Pharmaceutical Sciences, 2021, 14(02), 165–171

Publication history: Received on 02 December 2020; revised on 15 February 2021; accepted on 17 February 2021

Article DOI: https://doi.org/10.30574/gscbps.2021.14.2.0394

Abstract

Neem *Azadirachta indica* is a useful traditional medicinal plant growing in Nigeria, India, and America. The phytochemicals and the biopesticidal components present were ascertained. The results showed that saponins, steroids and terpenes were mostly present, while tannins and glycosides were moderately present, and alkaloids, flavonoids, phenols and oxalic acid were least present. The presence of these phytochemical could account for the therapeutic uses of neem.

Keywords: Biopesticidal; Neem; Phytochemicals; Therapeutic

1. Introduction

Neem plant is a native of India, where it is known as divine tree; "life giving tree". It belongs to *maliceae* family. Away from India, it is commonly found in Africa and America. It occurs naturally in tropical region and sub-tropical zones. However, it can still be planted or cultivated. Neem tree is an incredible therapeutic plant [1] that has been declared the tree of the 21st century by the United Nations [2].

The plant kingdom represents a rich store house of organic compounds, many of which have been used for medicinal purposes and could serve as a lead for the development of novel agents having good efficacy in various pathological disorders in the coming years. Neem plant is considered to be the richest sources of drugs for traditional medicine, modern medicine, nutraceuticals, food supplements, folk medicine, pharmaceutical intermediates and chemical entities for synthetic drugs [3]. Some of the phytochemicals contained in Neem plant have been isolated, quantified and identified through Intensive studies. These bioactive chemicals have provided leads in the development of several life-saving drugs, which are in use today [4]. Extract from *Azadiracta indica*, which is referred to as dogonyaro in some parts of Nigeria are mostly recommended in ancient medical texts. The leaves can be used as drug for diabetes, eczema and fever. Thus, the objective of this research was to ascertain the phytochemical constituents of neem plant and relate it to some of its traditional use.

*Corresponding author: Innocent Izuchukwu Ujah
Department of Applied Biochemistry, Faculty of Applied Natural Sciences, Enugu State University of Science and Technology, Enugu, Enugu State, Nigeria.

2. Material and methods

2.1. Collection of sample

Fresh leave of Neem Plant were collected on 15th of July 2018, from Agbani farm in Nkanu West Local Government Area of Enugu State, Nigeria. It was identified by Prof. Helen Nwamba, a Botanist of Enugu State University of Science and Technology.

2.2. Sample preparation

It was air dried at room temperature three weeks after which, it was taken to electric grinding machine where the engine was properly washed and dried to avoid contamination. The leave was ground to required texture.

2.3. Extraction

For aqueous extraction, the weighing balance was zeroed and the filter papers were placed on the weighting balance, a quantity 0.5 g of the sample were weighed and poured into a reagent bottle. 10 ml of distilled water was added to soak and facilitate extraction. After some minutes, proper filtration was carried out using filter paper after which the filtrate was tested with several reagents to determine the presence and quantity of tannins, alkaloids, saponins glycosides, terpenoids, flavonoids, steroids and phenols.

For ethanol extraction, the weighing balance was zeroed and the filter paper were place on weighting balance. The sample was weighed 0.5 g into 250 ml beaker; 100 ml of 10 % acetic acid in ethanol was added to the sample and covered. In ethanol extraction it was done in closed system for proper extraction.

2.4. Test for tannin

This is based on utilization of standard conventional protocols as illustrated by [5]. A quantity, 0.5 g of sample was weighed out and stirred with 10 ml of distilled water and then filtered. To the 2 ml of filtrate measure out in the test tube, few drop of 1% ferric chloride solution were added formation of blue green precipitate was confirmed indicating the presence of tannins.

2.5. Phytochemical Screening

2.5.1. Test for alkaloids

The method of [6] was adopted in testing for alkaloids in neem plant. A quantity of 0.5 g of sample was dissolved in hydrochloric acid and filtered using filter paper to the 2 ml of filtrated was treated with dragendroff's reagent (solution of potassium Bismuth iodide) formation of red precipitate confirmed indicating the presence of alkaloid the test is called dragendroff's test. To 2 ml of filtrates was treated with Hager's reagent, formation of yellow colour confirmed the presence of alkaloid.

2.5.2. Test for saponins

The method of [5] was adopted in testing for saponins in neem plant. A quantity of 0.5 g of sample was boiled with 50 ml of distilled water and filtered. To 5 ml of each filtrate, 3 ml of distilled water was added and shaken vigorously for about 5 minutes, formation of frothing was confirmed showing the presence of saponins.

2.5.3. Test for glycosides

The method of [7] was adopted in testing for glycosides in neem plant. A quantity of 0.5 g of sample was dissolved in ethanol for about 10 minutes for proper extraction and filtered. To 5 ml of each filtrate, 0.3 ml of Fehlings solution A and B was added until it turn to alkaline indicating the presence of glycoside.

2.5.4. Test for terpenoids

The method of [7] was adopted in testing for terpenoids in neem plant. A quantity of 0.5 g of sample was dissolved in ethanol for about 10minutes for proper extraction and filtered. To 5 ml of each filtrate was added 1 ml of acetic anhydride followed by addition concentrated H_2S0_4. A change in colour from pink to violet showed presence of terpenoid.

2.5.5. Test for flavonoids

The method of [6] was adopted in testing for flavonoids in neem plant. A quantity, 0.5 g of the sample dissolve in distilled water and filtered to 5 ml of filtration, 3 ml of lead ethanoate solution was added. Appearance of pale yellow-brown (buff-coloured) confirmed the presence of flavonoid.

2.5.6. Test for steroids

The method of [6] was adopted in testing for steroids in neem plant. A quantity of 0.5 g of the sample dissolved in distilled water and filtered to 4 ml of the filtrate, 2 ml of acetic acid was added and allowed the solution to cool well in refrigerator followed by the addition of concentrated H_2SO_4 carefully. Colour change from violet to bluish green indicated the presence of steroidal ring.

2.5.7. Test for phenol

The method of [6] was adopted in testing for phenol in neem plant. A quantity of 0.5 g of the sample was boiled with 15 ml of distilled water and filtered. To 2 ml of the filtrate, few drops of 10% ferric chloride solution were then added. Formation of violet colour was confirmed indicating the presence of phenolic hydroxyl group.

2.6. Quantitative Analysis

2.6.1. Determination of alkaloids

A quantity, 1.0 g of the powdered sample was weighed using electric weighing balance into a 250 ml beaker and 100ml of 10% acetic acid in ethanol. The mixture was allowed to stand for four hours for proper extraction totake place. The sample was filtered with filter paper and the extract was concentrated on a water bath to one quarter of the original volume. A volume, 20ml of ammonium hydroxide (NH_4OH) was added drop wisely to form precipitate of the alkaloid in the filtrate. The filtrate was weighed with the NH_4OH and filtered. After filtering, the filter paper and the precipitate was dried in an oven at 40 ^{0}C and weighed. The alkaloid content was determined using the following formula.

Concentration of Alkaloid = $\frac{W_2 - W_1}{W_3}$ Where,W_1 = weight of empty filter paper

W_2 = weight of the alkaloid and filter paper,W_3 = weight of sample used

2.6.2. Determinations of saponins

A quantity, 1.0g of the powdered sample was weighed using electric weighing balance into a 250 ml beaker and soaked with 100 ml of 20 % ethanol for three (3) minutes and heated for three (3) hours at 55 ^{0}C for proper extraction then filtered. The residue was re-extracted with another 100 ml of 20% ethanol. The two extracts were combined and heated to 40 ml at 90 ^{0}C on a water bath. The concentrate was transferred into a 500 ml separating funnel and 20ml of diethylether was added and shaken vigorously, the upper layer was discarded. The purification process was repeated and 60ml of n-batanol was added, the lower layer was discarded while the upper layer was collected. The combined n-butanol extract was washed with 10ml of 5% aqueous NaCI and the lower layer was discarded while the upper layer was collected in a weighed beaker and heated to dryness. The beaker was allowed to cool in a desiccators and re-weighed. The saponin content was determined using the following formula.

Concentration of saponin = $\frac{W_2 - W_1}{W_3}$ Where W_1 = weight of empty beaker

W_2 = weight of beaker + sample heating W_3 = weight of sample used

2.6.3. Determination of tannins

A quantity, 1.0 g of neem powder was weighed into a plastic bottle and 50ml of distilled water was added and shaken for 3 hours in a vibrator. The sample was filtered into a 50ml volumetric flask and made up to mark. A volume, 5ml of the filtrate was dispensed into a test tube and mixed with 2 ml of 0.1M $FeCI_2$ in 0.1NHCI and 0.008 M potassium ferrocyanide, the absorbance was measured at 720nm for 10 minutes. The tannin concentration was determined using the following relation.

Concentration of tannin = $\frac{Abs \times D.F}{1000 \times weight\ of\ sample\ used}$

Where, Abs = value of absorbance read, D.F = dilution factor

2.6.4. Determination of flavonoids

A quantity, 1.0g of the powdered sample was repeatedly extracted with 100 ml of 80% aqueous methanol at room temperature. The solution was shaken for 30minutes and filtrate was transferred into a weighed beaker and evaporated to dryness over a water bath and weighed again. The time for the first extraction was 1 hour, 45minutes for the second extraction and 30 minutes for the third extraction. Flavonoid was determined using the following formula.

Concentration of flavonoid = $\frac{W_2 - W_1}{W_3}$ Where, W_1 = weight of empty beaker. W_2 = weight of beaker + sample after drying W_3 = weight of sample used

2.6.5. Determination of steroids

A quantity, 1.0 g of the powdered sample was dispersed in 100ml of distilled water into a conical flask, the mixture was shaken for 3 hours and allowed to stand overnight. It was then filtered and the filtrate was eluted with 10ml normal ammonium hydroxide solution. A volume, 2ml of the elute was put into a test tube and mixed with 2 ml of chloroform and 3ml acetic hydride added to the mixture, followed by 2ml of concentrated H_2SO_4 drop wisely. The absorbance was measured in a spectrophotometer at 420 cm. The steroid concentration was determined using the following relationship.

Concentration of steroids = $\frac{Abs \times Path\ length}{100 \times weigh\ of\ sample\ used}$

2.6.6. Determination of terpenoid

A quantity, 0.1g of the extract was weighed out separately, macerated with 20 ml of ethanol and filtered through Whatman No.1 filter paper. The filtrates (1 ml) were pipette out and 1 ml of 5% phosphomolybdic acid solution was added and shaken. Gradually 1 ml of concentrated H_2SO_4 was added to each. The mixtures were left to stand for 30 minutes. A volume, 2 ml of ethanol was added and absorbance was measured at 700 nm.

Concentration of terpenoid $= \frac{Abs \times Path\ length}{100 \times weigh\ of\ sample\ used}$

2.7. Test for oxalic acid content

A standard solution of oxalic acid was prepared by dissolving 100 mg of oxalic acid ($C_2H_2O_42H_2O$ mol) in distilled water and diluted to 100 ml with distilled water. A quantity of the powdered sample 0.5 g was transferred to 50 ml volumetric flask to which 30 ml 0.25N HCL was added and kept in boiling water bath for about 15minutes. Cooled at room temperature and volume of solution was used as extract for determination of oxalic acid.

Assay mixture contained 2 ml standard oxalic solution at various concentrations, ranging from 0.25 to 1.50 mg per ml, prepared with 2 ml of sulfuric acid instead of oxalic acid solution. Then 2 ml of indole reagent was added in each test tube including blank, and the reagent to run down the side of the tube to minimize heat development. All test tubes were placed in water bath at 80 to 90^{o}c for 45minutes cooled to room temperature and absorbance was measured at 525nm in spectrophotometerno7525.

3. Results and discussion

The results of the qualitative analysis of neem plant (*Azadirachta india*) is shown in Table 1. A great deal of studies on phytochemistry has been conducted on plants recently, particularly on their leaves, bark, roots, seeds and stems [8]. These phytochemicals have been reported to have many biological and therapeutic properties and are extensively used in the drug and pharmaceutical industry.

Owing to the numbers of human challenges *Azadirachta indica* can meet, it has become an important plant [8]. Table 1 reveals that saponin, steroid and terpenoid are most present in aqueous extract of *A. indica* while tannins and glycoside are in moderate concentration, alkaloids, flavonoids, phenol and oxalic acid have low concentration.

Table 1 Phytochemical and biopesticidal content of neem plant (*Azadirachta india*).

Components	Abundance
Alkaloids	+
Saponins	+++
Tannins	++
Steroid	+++
Terpenoid	+++
Glycoside	++
Flavonoid	+
Phenol	+
Oxalic acid	+

+++: Most present; ++: Moderately present; +: Least present

These results bear similarities to ones obtained by [9], in their aqueous neem leaf extracts saponins occurred the most, tannins and glycoside are moderate while alkaloid, flavonoids and reducing sugars are low. In the analysis of their methanolic extract however, Saponin was very low while glycoside was present in highest concentration. In addition to the phytochemicals in this present study, [10] observed the presence of resins.

Much of the protective effects of herbal plants have been attributed to their phytochemicals constituents [11], alkaloids, flavonoids, glycosides, saponins for examples exert multiple biological effects like anti-inflammatory, anti-allergic, antioxidant, anti-diabetic, anti-viral and anti-cancer activities, anti-leprosy activities, antimicrobial activity.

Compare to synthetic drugs, use of bio-actives compounds of medicinal plants have several advantages which include fewer side effects, better patient tolerance, relatively less expensive and renewable in nature [12].

Alkaloids acts on diverse metabolic system in humans and animals, withalkaloids such as cocaine, the psychotic psilocin, nicotine, the analgesic morphine used as medications and recreational drugs [13, 14]. Glycosides play numerous important roles in living organisms and are used in medications. Many plants store chemicals in the form of inactive glycosides which can be activated by enzymes breaking off the sugar part and making the glycoside available for use [15]. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves and ginger, the yellow colour in sunflowers and the red colour in tomatoes [16].The steroids and sterols in animals are biologically produced from terpenoid precursors. Through isoprenylation terpenoids are added to proteins toenhance their attachment to the cell membrane. Flavonoids have inhibitory activity against organisms that causes plant diseases such as *Fusarisiumoxysporum*. In higher plants, flavonoids are involved in UV filtration, symbiotic nitrogen fixation and floral pigmentation. They may also act as chemical messengers, physiological regulators and cell cycle inhibitors. Steroid and their metabolites are frequently used as signaling molecules. Steroids along with phospholipids function as components of cell membranes with steroids such as cholesterol decrease membrane fluidity [17].Tannins are widely distributed in many species of plants, where they play a role as pesticides and plant growth regulation [18]. Resins made of tannins have been investigated to remove mercury and methyl mercury from solution [19]. Immobized tannins have been tested to recover uranium from seawater [20]. Phenol derivatives are also used in the preparation of cosmetics including sunscreens [21], hair colorings and skin lightening preparations. Concentrated phenol liquids are commonly used in the surgical treatment of in grown toenails to prevent a section of the toenail from growing back, process is called phenolization. In plants, saponins may serve as anti-feed ants and to protect against microbes and fungi [22]. Saponins are toxic to cold-blooded organisms and insects at particular concentrations [23].

4. Conclusion

Azadirachta indica is a good source of phytochemicalst, the study has shown the presence and concentration of phytochemicals of neem plant. These phytochemicals have pharmacological functions which accounts for the use of neem leaves in traditional medicine and pest control.

Compliance with ethical standards

Acknowledgments

The author wish to acknowledge Head of Department Applied Biochemistry ESUT, the Technologists in the Departmental Laboratory for their support during the research for this work

Disclosure of conflict of interest

Manuscript title: Phytochemicals of neem plant (*Azadirachta indica*) explains its use in traditional medicine and pest control

The authors whose names are listed immediately below certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript

Innocent Izuchukwu Ujah, Chukwunonso Anthony Nsude , Onuabuchi Nnenna Ani, Uchenna Blessing Alozieuwa, Innocent Oluwaseun Okpako and Amos Ejiofor Okwor

References

[1] Kumar S, Vandana UK, Agrwal D, Hansa J. Analgesic, Anti-inflammatory and Anti-pyretic Effects of *Azadirachta indica*(Neem) Leaf Extract in albino rats. *International Journal Scientific Research.* 2015;4: 713–721.

[2] Dhama K, Mani S, Chukrubarty S, Tiwari R, Kumar A, Selvaraj P, Rai RB. Herbal Remedies to combat Cancers in Humans and Animals. *A Review International Journal of Current Research.* 2013; *5*: 1908-1919.

[3] Mahima AK, Verma A, Kumar A, Rahal R, Deb SK. Immumomodulatary Therapeutic Potential of Herbal, Traditional and Ethnovertinary Medicine. *Pakistan Journal of Biological Science.* 2013;15: 754-774.

[4] Padal SB, Sandhya B, Chandrasekhar P, Vijayakumar Y. Folklore treatment of skin diseases by the tribes of Madugula Mandalam, Visakhapatnam District, Andhra Pradesh, India. *Journal of Environmental Science, Toxicology and Food Technology.*2013;4: 26-29

[5] Harborne JB. *Textbook of Phytochemical Methods. A Guide to Modern Techniques of Plant Analysis* (5th ed.). London: Chapman and Hall Ltd. 1998.

[6] Trease GE, Evans WC. *Pharmacognosy* (11th ed.). London, UK: Macmillan Publishers. 1989.

[7] Sofowora A. *Medicinal Plants and Traditional Medicine in Africa* (2nd ed.). Ibadan, Nigeria: Spectrum Books Ltd. 1993.

[8] Kala S, Johnson M, Raj I, Bosco D, Jeeva S. Preliminary Phytochemical Analysis of Some Medicinal Plants of South India. *Journal of Natura Conscientia*. 2011; *2*: 478-481.

[9] Dash SP, Dixit S, Sahoo S. Phytochemical and Biochemical Characterizations from Leaf Extracts from *Azadirachta indica*: An Important Medicinal Plant. *Biochemistry and Analytical Biochemistry*. 2017;*6*: 323.

[10] Itelima JU, Nwokedi VC, Ogbonna AI, Nyam MA. Phytochemical screening and antimicrobial activity evaluation of aqueous and ethanolic extracts of the leaf of *Azadirachta indica* Juss (neem) on some microorganisms. *World Journal of Microbiology*. 2016; *3*(1): 56-60.

[11] Singh V, Chauhan D. Phytochemical evaluation of aqueous and ethanolic extract of neem leaves (*Azadirachta indica*), *Indo American Journal of Pharm Research.* 2014; 4:5943-5948.

[12] Vermani K, Garg S. Herbal medicine for sexually transmitted diseases and AIDS. *Journal of Ethnopharmacology*. 2002; 80: 49-66.

[13] Raymond S, Jonathan S, Jah J, Michael W. *Biochemistry of Alkaloids.* 2010; 15:83-90

[14] Rhoades DG. Evolution of plants chemical defense against herbivoures in Rosenthal. Their interaction with Secondary Plant Metabolites. 2010; 2:1-45.

[15] Lindhorst, *TK. Essentials of Carbohydrate Chemistry and Biochemistry. Wiley-VCH. 2007;* ISBN 978-3-527-31528-4.

[16] Michael, S. A life of its own. 2009; The New Yorker.

[17] Sadava *D,* Hillis *DM, Heller HC,* Berenbaum *MR. Life: The Science of Biology 9th Edition. San Francisco: Freeman. 2011; pp. 105–114.*

[18] Katie, Jr. EF, Thorington RW. Squirrels: The Animal Answer Guide. Johns Hopkins University Press, Baltimore, London, 2006; 91.

[19] *Torres J, Olivares S, De La Rosa D, Lima L,* Martínez *F, Munita C S, Favaro DIT. "Removal of mercury(II) and methylmercury from solution by tannin adsorbents". Journal of Radioanalytical and Nuclear Chemistry. 1999;* ***240*** *(1): 361–365.*

[20] *Takashi Sakaguchia; Akira Nakajimaa. "Recovery of Uranium from Seawater by Immobilized Tannin". Separation Science and Technology. 1987;****22*** *(6): 1609–1623*

[21] Svobodová A, Psotová J, Walterová D. Natural Phenolics in the Prevention of UV-induced Skin Damage. Biomed Papers, 2003; 147:137–145

[22] Foerster, *H. MetaCyc Pathway: Saponin Biosynthesi. 2006.*

[23] Liener, *IE . Toxic constituents of plant foodstuffs. The Proceedings of the Nutrition Society. New York City: Academic Press. 1980; 29:pp. 56–7*