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Phytochemical and Biochemical Characterizations from Leaf Extracts from *Azadirachta Indica*: An Important Medicinal Plant

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Abstract

Throughout history, human civilizations have kinetically circumvented plants which have influenced a lot to the humanity. Plants have the facility to endanger diverse variety of phytochemical and biochemical compounds which can be acclimated to perform different biological functions. Many of these phytochemicals have salutary effects on long-term health when consumed by the human and can be efficaciously used to treat human diseases. The current research paper deals with the various phytochemical and biochemical analysis of *Azadirachta indica*. The analysis was carried out utilizing standard methods and protocols. Phytochemical analysis of methanolic leaf extracts of *Azadirachta indica* has shown the presence of biological compounds like, Alkaloids, Flavonoids, Saponins, etc which are then compared to aqueous leaf extracts of the plant. Biochemical analysis includes the estimation of chlorophyll content, carbohydrate content and proline content. The result suggests that the *Azadirachta indica* extracts contain plenty of phytochemicals with antimicrobial, anti-inflammatory and antioxidant properties.

Keywords: *Azadirachta indica*; Medicinal plant; Solvent extraction; Phytochemical analysis; Biochemical analysis

Introduction

Since ancient time, people are exploring the plant species in search of new drugs, which has resulted in exploitation of large number of medicinal plants with curative properties to treat various ailments. The importance of medicinal plants becomes more patent now in developing countries. In India, it is estimated that 80% of population depends on plants to therapy themselves, of those about 60% populace use medicinal plants habitually to battle certain ailments and almost 40% human use such plants in pharmaceutical industries [1]. The World Health Organization (WHO) has outlined herbal medicine as culminated labeled medicinal products that incorporate lively ingredients as aerial or underground accessories of plants or other plant fabric [2]. Neem (*Azadirachta indica*) is a tree within the Meliaceae family. Neem is also known as 'arista' in Sanskrit-A word that suggests 'ultimate, entire and imperishable'. Fruit, seeds, oil, leaves, roots, bark and just about each part of the tree is bitter and contain compounds with verified antiviral, anti-inflammatory, anti-ulcer and antifungal, antiplasmiodal, antiseptic, antipyretic and anti-diabetic houses [3]. The Chemical components incorporate many biologically energetic compounds that can be extracted from neem like alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones. Azadirachtin is authentically an amalgamation of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more efficacious [4]. The *Azadirachta indica* leaves are greatly used among the quite a lot of tribes of India to remedy cuts, wounds and different minor dermis illnesses [5,6].

World Health Organization reported the investigation of Neem with emboldening results on the therapeutic treatment of HIV and cancer. Consequently, such plants should be investigated deeply to better understand their properties, safety and efficacy. Thus, because the plant possesses immensely colossal meditative properties, the aim of this study correlates the phytochemical and biochemical attributes present within the aqueous and methanol extracts of the leaves of *Azadirachta indica*. However, be aware of potential side effects associated with neem leaf before starting any type of treatment involving the herb. The appropriate dose of neem depends on several factors such as the user's age, health, and several other conditions. Presently, there is not enough scientific information to determine an appropriate range of doses for neem. Keep in mind that natural products are not always necessarily safe and dosages can be important. Be sure to follow relevant directions on product labels and consult your pharmacist or physician or other healthcare professional before using. Excess uses of neem may cause common side effect that you can experience with the consumption of neem leaf is an increase in fatigue, liver or kidney disease, Reye's syndrome symptoms in infants, stomach ailments and disorders, ranging from diarrhea to indigestion, shortly after neem leaf consumption or use.

Materials and Methods

Preparation of extract

Fresh leaves of *Azadirachta indica* (Family: Meliaceae) was accumulated locally from green house of Tectona Biotech Resource Center (TBRC), Bhubaneswar. The Leaves was air-dried and grinded into fine powder. Five gram of grinded powder had been percolated with one 200 ml of solvent (Methanol and aqueous) for extraction and kept at soxhlet 150°C temperature for 36 hours. After extraction, the extracts obtained were filtered and concentrated. Then the extract used to be taken for phytochemical evaluation

Phytochemical screening

The extracts were analyzed by the following procedures to test for the presence of the alkaloids, glycosides, flavonoids, reducing sugars, Terpenoids, saponins, tannins [7]. The powdered leaf was extracted with the required solvent and the obligatory reagent was integrated to the right quantity of the extract. All observations were then recorded.

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Test for alkaloids: The extract of *Azadirachta indica* was evaporated to dryness and the residue was heated on a boiling water-bath with 2% Hydrochloric acid. After cooling, the mixture was filtered and treated with a few drops of Meyer's reagent 10. The samples were then observed for the presence of turbidity or yellow precipitation.

Test for glycoside: 2 ml of test solution, 3 ml of glacial acetic acid and 1 drop of 5% ferric chloride were integrated in a test tube. 0.5 ml of concentrated sulphur acid was integrated by the sides of the test tube. Formation of blue color in the acetic acid layer indicates the presence of cardiac glycosides.

Test for flavonoid: 4 mg of extract solution was treated with 1.5 ml of 50% methanol solution. The solution was warmed and metal magnesium was integrated. To this solution, 5-6 drops of concentrated hydrochloric acid was integrated and orange or red color was observed for presence of flavonoids.

Test for reducing sugars: To 0.5 ml of extract solution, 1 ml of dihydrogen monoxide and 5-8 drops of Fehling's solution was integrated at boiling and observed for brick red precipitate.

Test for saponins: 5 ml of extract was shaken vigorously with 5 ml of distilled water in a test tube and heated. The formation of stable foam was accepted as an indication of the presence of saponins.

Biochemical analysis

The biochemical analysis of the extract of *Azadirachta indica* leaves proline estimation, carbohydrate estimation and chlorophyll estimation was carried out using Ninhydrin Test, Anthrone's method and Arnon method respectively.

Estimation of proline: Proline content was measured using ninhydrin reagent. Fresh sample (0.5 g) was homogenized in 10 ml of 3% aqueous sulfosalicylic acid and centrifuged at 9000 rpm for 15 min. Supernatant (2 ml) was mixed with 2 ml acetic acid ninhydrin and incubated for 1 h at 100°C. The reaction was terminated in ice bath. The product was extracted with 4 ml of toluene by vertexing for 20-30 sec and absorbance was read at 520 nm against toluene. Proline content was measured using standard curve of Pure Proline Figure 1.

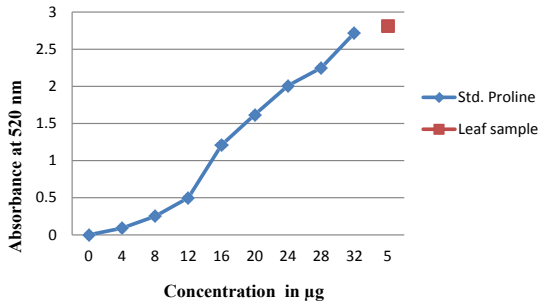

Figure 1: Standard curve of proline with neem sample.

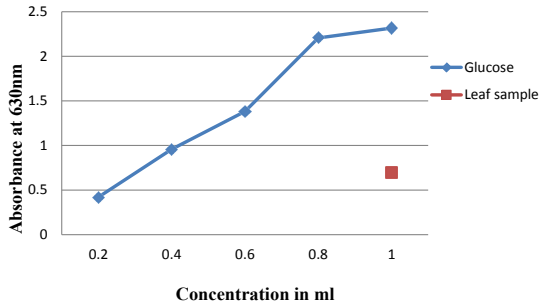

Figure 2: Standard curve of glucose with neem sample.

Estimation of carbohydrate: Total soluble carbohydrates were estimated quantitatively by using Anthrone's method. Total soluble carbohydrate was calculated with the help of a reference curve using D-glucose as standard. The reducing sugar content was estimated following the method of Lindsay and the OD was recorded at 630 nm Figure 2.

Estimation of chlorophyll: 100 mg of leaf was taken and chlorophyll was extracted in diffused and deem light with 10 ml of acetone using small glass mortar and pestle. The extracts were pooled together and centrifuged at 5000 rpm for 15 minutes and the OD was recorded at 645 nm and 663 nm in spectrophotometer. The amount of chlorophyll was calculated by the formula $(20.2(A_{645}) + 8.02(A_{663}))$.

Results and Discussion

Phytochemical analysis

Azadirachta indica has become important in the global context today because it offers answers to the major concerns facing mankind. It is a fast-growing evergreen popular tree found commonly in India, Africa and America. As recorded in Table 1, methanolic extract of *A. indica* shows the presence of glycoside having highest concentration, while alkaloids, flavonoids, tannins and sugar having moderate concentration and saponins having low concentration.

At the same time, in aqueous extract was found to have maximum number of phytoconstituents in saponins and flavonoid, sugar have low concentration. The Medicinal plants are rich in secondary metabolites which include alkaloids, flavonoids, saponins and related active metabolites which are of great medicinal value and have been extensively used in the drug and pharmaceutical industry. These secondary metabolites are reported to have many biological and therapeutic properties. Recently number of studies had been reported on the phytochemistry of medicinal plants, particularly on the vegetative parts like leaves and stems etc [8-11]. Alkaloids, flavonoids, glycosides have been reported to exert multiple biological effects like anti-inflammatory, anti-allergic, antioxidant, anti-diabetic, anti-viral and anti-cancer activities, anti-leprosy activities, antimicrobial activity etc. The phytoconstituents are well known for its curative activity against several human problems such as ulcers, swollen liver, malaria, dysentery, diarrhea etc. A variety of herbs and herbal extracts contain different phytochemicals with biological action that can be of valuable therapeutic index. Much of the protective effect of herbal plants has been attributed by phytochemicals, which are the non-nutrient compounds [12].

Biochemical analysis

Estimation of proline: Proline also known as Marverick immino acid

Components	Test	Scoring	
		Aqueous	Methanol
Alkaloids	Mayer's reagent	+	++
Saponins	Frothing	+++	+
Tannins	Ferric choloride	++	++
Glycosides	Killer-Killiani test	++	+++
Flavonoids	Pew's	+	++
Reducing sugars	Fehling's	+	++
Key: + =low concentration, ++ =moderate concentration, +++ =high concentration			

Table 1: Phytochemical components of neem leaf aqueous and methanol extract.

Tube Number	Amount taken from stock	Distilled water (µl)	Glacial acetic acid (ml)	Ninhydrin reagent (ml)	Amount of Toluene (ml)	Concentration of Proline (µg)	Absorbance at 520 nm
Blank	0	2000	2	2	4	0	0
1	4 µl	1996	2	2	4	20	0.09
2	8 µl	1992	2	2	4	40	0.25
3	12 µl	1988	2	2	4	60	0.5
4	16 µl	1984	2	2	4	80	1.21
5	20 µl	1980	2	2	4	100	1.62
6	24 µl	1976	2	2	4	120	2.01
7	28 µl	1972	2	2	4	140	2.25
8	32 µl	1968	2	2	4	160	2.72
9	5 µl	1995	2	2	4	25	2.81
Test tube 1 to 8: Standard sample; Test tube 9: Plant sample							

Table 2: Standard graphs for proline.

Test Tube No.	Volume of Glucose (ml)	Volume of DDH20 (ml)	Anthrone Reagent (ml)	O.D. at 630 nm
0	0	1	4 ml	----
1	0.2	0.8	4 ml	0.421
2	0.4	0.6	4 ml	0.958
3	0.6	0.4	4 ml	1.382
4	0.8	0.2	4 ml	2.206
5	1.0	-----	4 ml	2.316
6	1.0	-----	4 ml	0.699
Test Tube 1 to 5: Standard sample; Test Tube 6: Leaf sample				

Table 3: Values to construct the standard graph for carbohydrate.

Sl. No.	Biochemical	Neem leaf in mg	Wavelength in nm	Reference	Optical Density
01	Chlorophyll	100 mg	645	80% Acetone	2.227
			663		1.766

Table 4: Estimation of chlorophyll in leaf extract of neem.

is the only amino acid with a secondary amine. It is unique in that the alpha-amino group is attached directly to the side chain, making the alpha carbon a direct substituent of the side chain. It is derived from L glutamate. The Proline analysis of Neem leaf extract taking glacial acetic acid as a standard showed very high amount of proline. As illustrated in Table 2 the absorbance of proline is very high i.e., 2.811 OD in 5 µl concentration of our sample at 520 nm wavelength. Proline is extremely important for proper functioning of joints and tendons. It is therapeutically important for neurodegenerative diseases like Alzheimer's and Parkinson's disease, Type 2 Diabetes Mellitus and Polycythemia Vera which is caused due to over production of RBC, WBC and platelets.

Estimation of carbohydrate: Carbohydrates are widely prevalent in the plant kingdom, comprising the mono-, di-, oligo-, and polysaccharides. Presence of Carbohydrate is generally not appreciated when the plant is considered for Therapeutics. In this present study, total soluble carbohydrate was estimated quantitatively by using Anthrone's method. Total soluble carbohydrate was calculated with the help of a reference curve using D-glucose as standard. The absorbance was taken 630 nm by using the UV-spectroscopy instrument. This was observed that 1.0 ml carbohydrate sample measured 0.699 OD at 630 nm which indicates the presence of comparatively lower content of carbohydrates Table 3.

Estimation of chlorophyll: Chlorophylls are light harvesting pigments integral to the photosynthetic process which allows plants to absorb energy from light. Chlorophyll concentration data provide information on a plants photosynthetic potential. Chlorophyll absorbs light most strongly in the blue portion of the electromagnetic spectrum, followed by the red portion. In our present study, the total chlorophyll content was estimated using Arnon method. 80% Acetone was taken as reference for Spectrophotometric determination. In the Spectrophotometric analysis, neem leaf sample showed different variance in different absorbance and the result is shown in Table 4. The absorbance Chlorophyll is measured as 2.227 at 645 and as 1.766 at 663. Based on these data the total chlorophyll content of the leaf extract comes to be 59.148 µg/ml.

Conclusion

Neem has a long history as a medicinal plant with diverse therapeutic applications. The phytochemical and biochemical experiments performed during the current study confirm that the extracts of the plant are rich in chlorophyll and proline. Methanolic extracts showed the presence of some common phytochemicals like alkaloids, saponins, tannins, glycosides, flavonoids and reducing sugars. Traditionally used by many people as an alternative treatment for a variety of health ailments and skin irritations, neem leaf extract is a common supplement you can find in most local supermarkets and drug stores. In line with the above findings it is suggested that the further research on Neem should be directed towards identification and quantification of active principles responsible for curing skin ailments and patenting of findings thereby making these accessible to mankind.

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