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PHYSICOCHEMICAL CHARACTERIZATION AND SPECTRAL CHARACTERIZATION OF NATURAL PRODUCTS (*AZADIRACHTA INDICA LINN STEM BARK*)

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ABSTRACT

The present study deals with Physicochemical Characterization and Spectral Characterization of Azadirachata indica (Linn). The stem were subjected to soxhalation using petroleum ether alcohol, water, chloroform, acetone and the extracts thus obtained were studied for Physicochemical Characterization screening various total ash value was found to be 3.47%w/w. the acid insoluble value and water soluble ash values were found to be 1.12% w/w and 4.2 %w/w respectively. The foreign organic matter was found to be 0.24% w/w where as the moisture content, foaming index and swelling index were found to be 14.085% w/w, less than 10 and 20 respectively, The water soluble extractive value was found to be 21.12% w/w. the ethanol soluble and ether soluble extractive values were found to be 18.32% w/w respectively, and FT-IR Spectral Characterization of ethanolic extract showed a number of components and different groups of the stretching values present.

Key Words: *Physicochemical, Azadirachta Indica, Extract, Spectral Characterization.*

INTRODUCTION

Neem (Azadirachta indica A. Juss) is a plant of the Meliaceae family belonging to the Indian subcontinent [1]. It was later introduced into many tropical countries of America and Africa including Senegal with a population of 18 to 30 million trees [2]. Different parts or extracts of neem have been used since a long time, particularly in traditional medicine [3]. Nevertheless, in Senegal, their properties and composition are still poorly understood and their potential is under-exploited. From the point of view of the added value, the neem seed is the most important part of the plant given its content in oil and its many active molecules. That is why special attention is paid to the seed. However, research on the seeds has increased since the isolation of azadirachtin as a natural insecticide [4]. Thus, many works have been carried out notably on their characterization. Studies have shown that the lipids content of the neem seed varies from 20 to 32% and the lipids content of the kernel varies from 30 to 52% [5-6]. Unfit for human consumption, it has multiple uses mainly for the soap, pesticide and pharmaceutical [7]. It also has antibacterial, antifungal and medicinal properties 8. Its fatty acids composition and sterols have been reported [9-10]. In addition to its oil composition, neem seeds contain more than 100 active compounds which are together called triterpenoids or limonoids, including azadirachtin that would be one of the most important biopesticides [10-12]. The average azadirachtin content of neem seed kernels can vary from 2.05 - 6.10 kg-1 [13]. Several studies concerning its extraction, its purification, its efficacy, its toxicity, etc. were conducted [14-17]. The proteins content of the seeds and its amino acids composition have also been reported [9].

India has encouraged scientific investigations of Neem tree as a part of its program to revitalize Indian tradition and also to increase commercial interest on Neem [19]. In India, this tree is called "Divine tree", "Wonder tree", "heal all", "Materia Medica", "Free tree of India", Nature's drugstore", "Village Pharmacy", "Panacea for all diseases" [20-22]. The Neem oil is isolated from its fruits and seed [18, 23-25]. Neem is the most important medicinal plant that has been declared as the "Tree of the 21st century" by the United Nations.

Thus as the plant possesses immense medicinal properties, the aim of the present study was to Physicochemical Characterization and Spectral Characterization of *Azadirachata indica (Linn).*

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MATERIALS AND METHODS

Collection of plant material:

The fresh Stem of *Azadirachta indica Linn (Neem)* were purchased from local nursery garden during the month of March 2016. The plant material was identified and authenticated at, Sri Krishna devaraya University, Botany Department by Dr. S. Thimma Naik. The fresh plant material was dried under shade. Dried plant material was powdered using mechanical grinder and passed through sieve no.60 to get the powder of desired coarseness. Powdered material was preserved in an air tight container.

Preparation of Plant extracts:

The bark was dried in the shed and coarsely powdered. The powder was extracted with ethanol in a soxhalet apparatus for 72h. The ethanolic extract was evaporated in vacuo giving the residue (24%). The ethanolic extract obtained was upended in distilled water in small amount and was extracted successively and exhaustively with petroleum ether, benzene, chloroform and acetone in the order of increasing polarity. The extract and fractions were concentrated in a rotary evaporator at reduced pressure.

Physicochemical characters:

Ash values:

The ash values were determined according to the methods prescribed in [26, 27].

Total ash:

About 2g of the powdered drug was weighed and spred as a fine layer at the bottom in a tared silica crucible. The crucible was incinerated at a temperature not exceeding 600^{0}C until free from carbon. The crucible was cooled and weighed. The entire procedure was repeated till a constant weight was observed. The percentage of the total ash was calculated with reference to the weight of the air dried drug.

Acid in soluble ash:

The ash obtained in the total ash, was boiled with 25ml of hydrochloric acid for 5min. the insoluble ash was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred to a tarred silica crucible together with ash less filter paper and ignited, cooled and weighed. The procedure was repeated till a constant weight was observed. The percentage of acid insoluble ash was calculated with reference to the air dried drug.

Water soluble ash:

The ash obtained as described in the total ash, was boiled with 25ml of hot water for 5 minutes and filtered on an ash less filter paper, washed with hot water. The insoluble ash was transferred to a tarred silica crucible and ignited at 600^{0}C.

The procedure was repeated to get a constant weight of the insoluble matter was subtracted from weight of the total ash. The difference in weight was considered for water soluble ash. The percentage of the water soluble ash was calculated with reference to the air dried drug.

Sulphated ash:

A silica or platinum crucible was heated to redness for 10 min, allowed to cool in a desiccators and weighed. 1 to 2g of the test drug substance accurately weighed was put into the crucible, ignited gently at first, until the substance was thoroughly charred, cooled and the residue moistened with 1ml of sulphuric acid. It was then heated gently until white fumes were no longer evolved and then ignited at 800+25^{0}C until all the black particles disappeared. The ignition was conducted in a place from air currents, the crucible allowed to cool, a few drops of sulphuric acid was added and ignited as before. It was allowed to cool and then weighed. The operation was repeated until two successive weighing did not differ by more than 0.5mb.

Nitrated ash:

A silica or platinum crucible was heated to redness for 10 min and the procedure as mentioned above in the determination of sulphated ash was carried out. Instead of sulphuric acid here nitric acid was used and rest of the procedure was same as mentioned.

Extractive values:

The extractive values were determined according to the methods prescribed in [26, 27]. Extractive value is a measure of the content of the drug and its nature. There are different types of extractive values.

Ethanol soluble extractive:

5g of previously weighed air dried drug was taken in a Stoppard flask and 100ml of 95% ethyl alcohol was added to it was shaken continuously for 4 h on an electric shaker. It was then filtered rapidly taking precautions against loss of solvent. 50ml of the filtrate was evaporated to dryness in a tared flat bottomed Petridis, dried at 105^{0}C and weighed. The percentage of ethanol soluble extractive was calculated with reference to the air dried drug.

Water soluble extractive:

5g of previously weighed air dried was taken in a Stoppard flask and 100ml of chloroform water was added to it. It was shaken continuously for 4 h on an electric shaker. It was then filtered rapidly taking precaution against loss of solvent. 50ml of the filtrate was evaporated to dryness in a traed flat bottomed Petridis, dried at 105^{0}C and weighed. The percentage of water soluble extractive was calculated with reference to the air dried drug.

Ether soluble extractive:

5g of previously weighed air dried drug was taken in a Stoppard flask and 100ml of ether was added to it. It was shaken continuously for 4 h on an electric shaker. It was then filtered rapidly taking precaution against loss of solvent. 50ml of the filtrate was evaporated to dryness in a tared flat bottomed Petridis, dried at 105^{0}C and weighed. The percentage of ether soluble extractive was calculated with reference to the air dried drug.

Foreign Organic Matter (FOM):

It is the material consisting of any or all of the following:

a) Parts of the organ or organs from which the drug is derived other than the parts named in the definition and description or for which the limit is prescribed in the individual monograph.

b) Any organs other than those named in the definition and description.

c) Matter not coming from the source plant and

d) Moulds, insects or other animal contamination.

Method: 100-500g of the original sample was spread was spread out in a thin layer. The sample was inspected with the unaided eye or with the use of a 6X lens and the foreign organic matter separated manually as completely as possible. The percentage of FOM from the weight of the drug taken was weighed and determined.

Determination of Moisture Content: (Loss on drying method)

Loss on drying is the loss in weight in percentage w/w determined by means of the procedure given below:

1g of drug was taken and powdered. A glass Stoppard, shallow weighing bottle that had been dried for 30 min under the same conditions to be employed in the determination was weighed. The sample put in the bottle, covered and contents accurately weighed. The sample was distributed as evenly as practicable by gentle sidewise shaking to a depth not exceeding 10 mm. the loaded bottle was placed in the drying chamber (Oven or desiccators), the stopper removed and it was left closed in the chamber. The sample was dried for the time specified in the monograph or to constant weight, so that two consecutive weightings did not differ by more than 0.5mg, the second weighing being made after one hour drying under specific conditions.

Determination of Swelling Index:

11g of the drug was transferred to a 100ml Stoppard cylinder containing 90ml of water. After shaking well for 30 seconds it was allowed to stand for 24 h, shaking gently on three occasions during this period. Sufficient water was added to produce 100ml. it was mixed gently for 30 seconds and allowed to stand for 5 h. the final volume was measured. The determination was repeated 3 times.

Determination of Faming Index:

About 1g of the plant material was reduced to a powder, weighed accurately and transferred to a 500mL conical flask containing 100mL of boiling water. It was maintained at moderate boiling for 30 minutes, cooled and filtered into a 100mL volumetric flask. Sufficient water was added through the filter to dilute the volume to 100mL. Place the above decoction was Placed into 10 Stoppard test tubes (ht 16 cm, dia 16mm) in a series of successive portions of 1,2,3 up to 10mL, the volume of the shaken in a lengthwise motion for 15 seconds, i.e., 2 frequencies per second. It was allowed to stand for 15 minutes and the height of the foam was measured.

Determination of Mucilage content:

5g of powdered drug was taken and 100mL of water added to obtain the aqueous extract of the powder. It was filtered through cotton and the mucilaginous filtrate collected. 10mL of this mucilage solution was mixed with 25mL of absolute alcohol to precipitate the mucilage. This mucilage precipitate was collected by filtration with a tared filter paper. The filter paper was dried along with the mucilage. After drying the filter paper was carefully weighed and mucilage content was calculated from the weight.

Fluorescence Analysis:

The fluorescence analysis was carried out by the method of [28-29]. Fluorescence of the stem of *Azadirachta indica Linn (Neem* was observed in day light and in UV (254nm) light using drug powder.

Analysis of the drug powder:

The drug powder was treated with different solvents in different test tubes. The solvents used were 1N Hydrochloric acid, 1N sodium hydroxide (aqueous), 1N sodium hydroxide (alcoholic) and 50% Sulphuric acid. Then they were subjected to fluorescence analysis in daylight and in UV light.

RESULTS AND DISCUSSION

Ash values:

The total ash value was found to be 3.47 %w/w. the acid insoluble value and water soluble ash values were found to be 1.12 % w/w and 4.2 %w/w respectively. The results were shown in **Table-1.**

Extractive values:

The results of the extractive values were shown in **Table-2.** The water soluble extractive value was found to be 18.12% w/w. the ethanol soluble and ether soluble extractive values were found to be 14.32% w/w and 3.42 % w/w respectively.

Other physic chemical parameters:

The results of the other physic chemical parameters like foreign organic matter, moisture content, bitterness value, foaming index and swelling index were shown in the **Table 3.** The foreign organic matter was found to be 0.24 % w/w where as the moisture content, foaming index and swelling index were found to be 15.85% w/w, less than 10.0 and 20 respectively. The results showed that the drug powder does not have any bitterness value and mucilage content.

Colour & consistency:

The colour, consistency and percentage yield of ethanol, petroleum ether, acetone, chloroform and water extract recorded in **Table 4.** Among the five solvent extract, the water extract has more yield compared with all the other solvent extracts.

Fluorescence Analysis:

The fluorescence analysis of drug of powder showed reddish brown color under white light and greenish brown color under UV light. The fluorescence analysis results of the extracts were presented in the **Table 5.**

As there is no standardization work on record of this much valued traditional drug, the present work was taken up with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant. In other words, the pharmacognostic features examined in the present study may serve as tool for identification of the plant for validation of the raw material and for standardization of its formulations.

Table No. 1: The ash values of stem bark of *Azadirachta indica Linn (Neem)*

S. No.	Parameter	Ash value (%w/w)
1	Total ash value	3.47
2	Acid insoluble ash value	1.12
3	Water soluble ash value	4.2

Table No. 2: The extractive values of stem bark of *Azadirachta indica Linn (Neem)*

S. No.	Type of extract	Extract value (%w/w)
1	Water soluble extract	18.12
2	Ethanol soluble extractive	14.32
3	Ether soluble extractive	3.42
4	Loss on drying	4.14

Table No. 3: The physico chemical parameters of stem bark of *Azadirachta indica Linn (Neem)*

S. No.	Parameter	Value
1	Foreign organic matter	0.24 %w/w
2	Moisture content	15.85 %w/w
3	Bitterness value	Nil
4	Foaming index	Less than 100
5	Swelling index	3.70
6	Mucilage content	Nil

Table No. 4: Colour & consistency of *Azadirachta indica Linn (Neem)*

S. No.	Extracts	Colour	Consistency	Yield % w/w
1	Ethanol	Green	Amorphous	18.2
2	Chloroform	Orange	Flakes	19.4
3	Acetone	Light green	Amorphous	17.5
4	Pet ether	Light yellow	Amorphous	12.5
5	Water	Light yellow	Sticky	22.5

Table No. 5: The Fluorescence analysis of the stem bark powder of *Azadirachta indica Linn (Neem)*

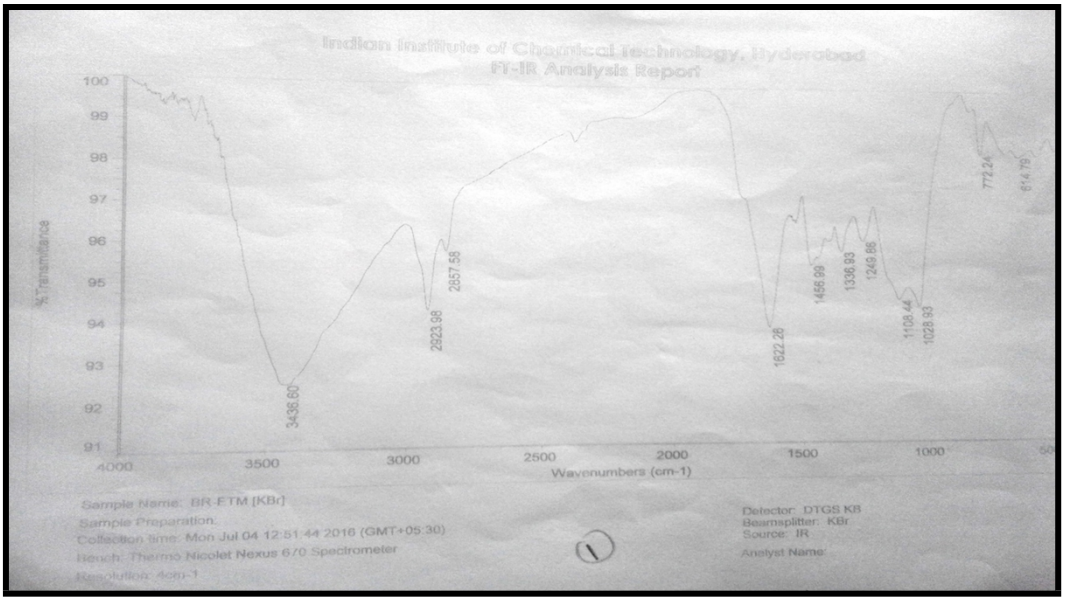
S. No.	Reagent	Long(366 nm)	Short (265 nm)	Day light
1	Powder + 50% H_2SO_4	Gray	Black	Brown
2	Powder + 50%HNO_3	Black	Green	Yellowish
3	Powder + 5%NaOH	Gray	Yellow	Yellow
4	Powder + 5%KOH	Dark Black	Whit	Pal Yellow
5	Powder + Methanol	_	yellow	Brown
6	Powder + Con H_2So_4	_	Black	Gray
7	Powder + Ammonia	Black	Gray	Brown
8	Powder + Con HNO_3	_	Green	Brown
9	Powder + Con HCl	_	Light Brown	Yellow
10	Powder + $FeCl_3$	Yellow	Green	Orange

Characterization:

Characterization of compound was carried out using following techniques [30].

FTIR spectroscopy:

FT-IR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extracts [31]. Furrier transformation – infra – red (FT – IR) spectrum results of shown in "Fig. 1". Several absorption peaks belonging to functional and / or structural groups were recorded. Appearance of broad band at 3399.2 cm-1 represents presence of stretching vibration of (-OH) hydroxyl group. The weak band at 2925 cm-1 indicates stretching vibration of aromatic (C-H) group and also the medium band at 1622 cm-1 represents bending vibration of aromatic (C=C) group.



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