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Pharmacognostical and Physicochemical Evaluation of the Leaves of *Azadirachta indica*

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ABSTRACT
Azadirachta indica L. (*A. indica*) commonly known as 'neem' is a member of the Mahagony family, widely available in Indian sub-continents. *A. indica* tree is popularly known for its vast therapeutic properties such as antimicrobial, antiparasitic, antidiabetic, insecticides and pesticides. The plant leaves are commonly used in several Ayurvedic formulations such as '*kwath*' and '*churna*.' Due to its high therapeutic value, it is important to standardize and document the quality parameters of the plant leaves.This study was aimed to investigate the pharmacognostical, phytochemical characteristic and fluorescence analysis of the leaves parts of *A. indica.*The leaves parts of the plant were subjected to macroscopical and microscopical characterization, followed by physicochemical evaluations using standardized procedures. The plant powder was subjected to fluorescence analysis in daylight and in ultraviolet-light (254, 365nm). Preliminary phytochemical screening of the ethanolic extract of *A. indica* was also performed to analyze the various phytoconstituents.In this study, the microscopic characteristics of *A. indica* were found to be consistent with earlier reports. The total ash value was relatively high (10.89% w/w). Alcohol soluble and water-soluble extractive values were found to be 14.07% and 17.98%, respectively. The result of fluorescence analysis showed that in day light, the plant powder exhibit various shades of green and brown fluorescence and various shades of green, black and blue were found in under UV light.Phytochemical analysis revealed the presence of alkaloids, naphthoquinones, carbohydrate, triterpenes, flavonoids, glycosides, saponins, proteins, and tannins in ethanolic extract of leaves of *A. indica*. These studies provided information for correct identification of this plant material. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.
Keywords: *Azadirachta indica*, Pharmacognostical, Phytochemical characteristic, Fluorescence analysis, Ayurvedic formulations, Microscopical

Introduction

India has a great wealth of traditional knowledge and wisdom. The classical Indian texts include *Rigveda*, *Atharveda*, *CharakSamhita*, and *Sushruta Samhita* [1]. Ayurveda is one of the traditional systems of medicine practiced in India and Srilanka and its origin traced back to 6000 BC. Ayurvedic medicines are largely based on herbal and herbomineral preparations and have specific therapeutic principles [2]. Traditional healers used different parts of medicinal plants as medicine. Among the different plant parts, leaves were more frequently used apart from whole plant parts, fruits, stems, roots, flowers, and latex. The methods of preparation fall into four categories, namely plant parts applied as a paste (38%), juice from fresh plant parts (20%), powder from dried plant parts (20%), some fresh plant parts (6%), and decoction (12%). Both external applications (for skin diseases, snakebites and wounds) and internal consumption of the preparations were involved in the management of diseases [3].Today, several medicinal plants and their products are in use, being employed as home remedies, over the counter drugs as well as raw materials for the pharmaceutical industry and they represent a substantial proportion of the global drug market [4]. The objective of producing inexpensive, potent and safer drugs of plant origin can be met to some extent by promoting compound formulations of plant medicines in their natural or

semi- processed form (powder or extracts) as used in traditional medicine for common disorders [5]. It becomes extremely important to make an effort toward standardization of the plant material being used as medicine. The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials are an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in the standardization of plant material include identification of its morphological, anatomical and biochemical characteristics [6].*Azadirachta indica* A Juss.
(Meliaceae) is one of the most important medicinal trees, which is used to treat different diseases in Unani System of Medicine as well as traditional system of medicine (Ayurveda, Homeopathic China and European Materia Medica). It is typically grown in tropical and sub-tropical region. Neem tree is one of the fast growing trees that can reach a height of 15-20 mts. In neem tree every part contributes high medicinal values like leaf is used to cure leprosy, eye problems, intestinal worms, anorexia, and skin ulcer. Bark is used to cure analgesic, alternative and curative of fever. Fruit is used to cure various diseases like intestinal worms, urinary disorder, eye problems, diabetes, wound and leprosy. Twig is used against cough, asthma, piles, urinary disorder and diabetes. Gum is effective against skin disease like ring worms, scabies wound and ulcers. Seed pulp is used to cure Leprosy and intestinal worms. Moreover, the root, bark, leaf, flower and fruit together is responsible for blood morbidity, itching, skin ulcer, burning sensation and leprosy respectively [7]. Neem flower is considered as one of the best medicinal plants due to its several therapeutic uses *viz*., bile suspension, elimination of intestinal worms and phlegm [8]. The *A. indica* flower contain chemical constituents like flavanones (flowerine and flowerone), sesquiterpenes, aromatic compounds, fatty acids, fatty acid esters, steroids, few hydrocarbons, azharone, azadirone, isoazadironolide and triterpenoids (Omethylazadironolide and diepoxyazadirol) [9]. The oil extracted from flowers of the tree also possessed cubebene, copaene, humulene, δ-cardinene, and a number of sesquiterpenes, which were responsible for antimicrobial activities [10]. Due to the presence of various bioactive components, it can be used to treat against various diseases like anorexia, nausea belching and intestinal worms [11]. Anthraquinone fraction of dried flower is taken orally for leprosy and hot water extract of flower is taken orally as an anti- hysteric remedy and used externally to treat wound and the extracts from young flower have strong antioxidant potential. An indicator of oxidative stress, malondialdehyde (MDA) was reduced by 46.0% and 50.6% for flower extracts [12]. Furthermore, the flowers of the tree are used as astringent and anthelmintic agents [13].Based on the foregoing, the pharmacognostic investigation, phytochemical analysis and fluorescence analysis of the leaves parts of *A. indica* have been done with a view to provide pertinent information on its identification, chemical elaboration, andpharmacological potential. Findings from this study would be useful as standards for the species as well as a source of reference for further scientific investigation of the species.

Materials and methods

Plant materials

The leaves of *Azadirachta indica* were collected from Bhopal Region, Madhya Pradesh, India in the month of Jan-Apr. 2019. The identification and authentication of plant was done by Dr. Saba Naaz, Botanist, from the Department of Botany, Saifia College of Science and Bhopal. A voucher specimen number **246/Saif./Sci./Clg/Bpl** was kept in Department of Botany, Saifia College of Science, Bhopal for future reference.Fresh leaves of plants were used for pharmacognostical studies. Leaves of *A. indica* were dried under shade and powdered to 60# separately and stored in airtight containers and used for phytochemical and pharmacological studies. The leaves were initially separated from the main plants body and rinsed with distilled water and shade dried and then homogenized into fine powder and stored in air tight bottles.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), SigmaAldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Plant characterization

Macroscopical characterization

The macroscopical description of *A. indica* leaves include size, shape, nature of outer and inner surfaces, types of fracture, and organoleptic characters like color, odour, taste etc. were studied [14].

Fluorescence analysis in dried powder

Powdered drug of different parts of plant gave different fluorescence under ultraviolet radiation.A small quantity (1 gm) of dried and finely powdered plant was treated with freshly prepared acids, alkaline solutions and different solvents. The drug powders were treated with acids viz., 1N HCl, Conc. HCl, 50% H2SO4, Conc. H2SO4, 50% HNO3, Conc. HNO3, picric acid and acetic acid. The drug powders were treated with alkaline solutions viz., aqueous NaOH and alcoholic NaOH. The drug powders were treated with different solvents viz., acetone, benzene, chloroform, petroleum ether, methanol, ethanol etc. They were subjected to fluorescence analysis in daylight and in short UV- light (254 nm) and long UV- light (365 nm).

Microscopical study

Transverse section of crude drug: The transverse sections were taken by placing the leaves between the thumb and four finger of the left hand. Using sharp razor held in the right hand, thin section was made the razor across the object in quick successions. The sections were transferred
in to watch glass containing water, added chloral hydrate to these sections, boiled, filtered and the sections were stained with phloroglucinol and hydrochloric acid (1:1) and the same was mounted in glycerin and observed under low power. In order to supplement the descriptive part, photomicrographs in different magnification of all necessary cells and tissues were taken. For the purpose of studying crystals and starch grain photographs of unstained slide were taken. For normal histological purpose, sections were photographed under the bright field light.

Powder microscopy: The shade dried leaves powder was powdered and the powders passed through sieve no. 60# separately and individually then subjected to powder analysis. Each one of the three powders was taken to which few drops of chloral hydrate was added and heated for one to two minutes. The chloral hydrate was used to clear the tissues and for clarification. To the cleared powder, a few drops of 1:1 mixture of phloroglucinol and HCl was added and then it was finally mounted with glycerin. Lignified tissue acquired pink colouration. In order to study starch grains, powder were mounted with water and one to two drops of dilute iodine, while to observe the calcium oxalate crystals unstained sections were mounted only with water.

Physicochemical evaluations

Physicochemical parameters of the powdered drug were determined and reported as total ash, water-soluble ash, and acid-insoluble ash values. Alcohol and water-soluble extractive values were determined to find out the amount of water and alcohol soluble components. The moisture content, foaming indexand pH were also determined by standard procedures [14, 15].

Extraction

Plant material fattening

Plant matter from *A. indica*was crushed up and allowed to air dry at ambient temperature. Soxhlation was used to remove the substance from the shade-dried plants using petroleum ether after it had been coarsely crushed up. The substance was extracted repeatedly until it had been adequately fatted.

Extraction by soxhlation process

*A. indica*powder that has been defatted was thoroughly extracted with ethanol using the soxhlation

process. The extract evaporated beyond their boiling points. The dried crude concentrated extract was weighed in order to calculate the extractive yield. When ready for analysis, it was then put into glass vials (6 x 2 cm) and stored in a refrigerator 4°C [16].

Phytochemical screening

According to the protocols described, phytochemical screening was done to find any bioactive compounds [17, 18]. By visually seeing a colour change or the production of precipitates following the addition of specific reagents to the solution, the tests were recognized.

TLC of plant extracts

TLC of ethanolic extracts of the leaf were performed using precoated silica gel TLC plates (Merck) as a stationary phase and toluene: ethyl acetate: acetic acid (7: 2.5: 0.5) as a mobile phase. The solvent was allowed to run up to 9 cm. The plate was observed as such under ultra-violet light (366nm). Rf values of number of fluorescent resolved spots were calculated.

Results and Discussions

The WHO has long recognized that traditional medicinal plants could be useful in an integrated health care delivery system of a country. Medicinal plants are starting material for any herbal preparation such as herbal medicines, herbal teas, and herbal oil. In developing countries, large numbers of the population are unable to afford pharmaceutical drugs, and they continue to use their own systems of indigenous medicine that are mainly plant based. These preparations are being used worldwide due to their therapeutic potential and as they are considered to be safe as compared to allopathic medicines. Hence, there is a great need to harness scientific and clinical research to investigate the quality, safety and efficacy of these herbal therapies [19]. These plants must not be dangerous, be effective and the preparations should not be adulterated or made harmful by parasites and microorganisms [20]. Thus for medicinal plants to be used alongside modern medicine, careful phytochemical, pharmacological and toxicological standardization of the chosen plants must be instituted so that dosage levels can be described in an informed way [21]. It was in this vein that the phytochemical, physicochemical and pharmacognostical investigations of the medicinal plant *A. indica* were pursued. *A. indica* is a medium-sized tree, reaching 15 to 30 m in height, with a large rounded crown up to 10-20 m in diameter. It is mainly evergreen but sometimes shed its leaves during the dry season. *A. indica* leaves are opposite, simple pinnate leaves are 20 to 40 cm long, with 20 to 30 medium to dark green leaflets about 3 to 8 cm long. The leaf petioles are short. Young leaves are reddish to purplish in colour. Leaf margins are toothed. *A. indica* flowers are white and fragrant that arises from the junction of the stem and petiole. They are normally in drooping flower clusters (panicles) which are up to 25 cm long. The results of organoleptic studies of *A. indica* presented in Table 1 &Figure1.The result of fluorescence analysis was summarized (Table 2). The powder from the leaf fluoresced green under daylight and short UV-light (254 nm), black under long UV-light (365 nm). The leaf of *A. indica* showed the characteristic fluorescent green treated with dil H2SO4, dil HCl, Conc. HNO3, Conc. H2SO4, ethanol, Dil. NH3, Glacial acetic acid, under short UV-light (254 nm).A transverse section of the leaf is biconvex. The upper and lower epidermis consists of polygonal cell carrying glandular and non-glandular trachoma's. Both upper and lower epidermis covered with smooth cuticle. The lamina has dorsiventral structure with one row of upper palisade being discontinuous in the midrib region. The midrib is prominent on the both surfaces showing subepidermal collenchyma's, cortical tissues and large central collateral vascular bundle. Cluster crystals of calcium oxalate are scattered in the cortical tissue as well as in the mesophyll. Secretory glands and mucilage cells are also, present in the parenchymatous tissues. The stomata are anisocytic type. The endodermis is well differentiated containing starch granulesFigure 2.Powder microscopy of *A. indica* powder showed simple fiber, crystals of calcium oxalate, xylem vessels, spongy parenchyma, epidermal cell, glandular and non glandular trichomes with chloral hydrate 10%. Crystals of calcium oxalate, glandular and non-glandular trichomes with glycerine 50% parenchymatous cell, epidermal cell with iodine 5%Figure 3.This study on physicochemical characteristics and preliminary phytochemical screening provides useful information which may help in authenticating the genuine plant along with the nature of phytoconstituents present in it [22]. There is a need to evaluate herbal treatments

by clinical trials using currently accepted protocols. Results for physicochemical parameters are given in Table 3. The total ash value was relatively high (10.89% w/w) which may be due to high content of phosphates, carbonates, silicates, and silica. The ash value is an important quantitative tool used to determine the authenticity and purity of drug. Percent weight loss on drying or moisture content was found to be 8.75%. The less value of moisture content could prevent bacterial, fungal, or yeast growth. Alcohol soluble and water-soluble extractive values were found to be 14.07% and 17.98%, respectively. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, saponins, and sterols. The successive extracts of leave parts of *A. indica*have revealed the presence of alkaloids, naphthoquinones, carbohydrate, triterpenes flavonoids, glycosides, phenols, saponins, proteins and tannins (Table 4). Thus, the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds [23]. TLC of extracts of *A. indica* were performed using precoated silica gel TLC plates (Merck) as a stationary phase and toluene: ethyl acetate: acetic acid (7: 2.5: 0.5) as a mobile phase. The plate was observed as such under ultra-violet light (366 nm)
Figure 4.

S,NO	**Parameters**	***A. indica***
1	Shape	Simple pinnate leaves
2	Position on stem	opposite
3	Size	7-10 cm length, 3-5 cm width
4	Odour	Odorless
5	Taste	Bitter
6	Colour	green
7	Foreign organic matter	Nil

Table 1: Organoleptic identification of *A. indica* leaves

Table 2: Fluorescence characters of powdered leave of *A. indica*

Leaves powder treated with different chemical reagents	**Day light**	**Long wave length**	**Short wave length**
Conc. HCl	Green	Black	Green
Dil. HCl	Green	Green	Green
Conc. H2SO4	Green	Black	Green
Dil. H2SO4	Green	Green	Green
Conc. HNO3	Green	Black	Green
10% NaOH	Color less	Color less	Color less
Ethanol	Green	Black	Green
Dil. NH3	Green	Black	Green
Barfoed`s reagent	Blue	Black	Blue
Glacial acetic acid	Green	Green	Green
Mayer reagent	Color less	Black	Color less
Dragendorff's reagent	Color less	Color less	Color less
Wagner reagent	Reddish brown	Black	Reddish brown
Hager reagent	Yellow	Black	Yellow

Table 3: Physicochemical parameters of *A. indica*

S. No.	Physicochemical parameter values (% w/w)	*A. indica*
1	Total ash	10.89
2	Water soluble ash	6.35
3	Acid insoluble ash	1.78
4	Loss on drying	8.75±0.23
5	Alcohol soluble extractive	14.07
6	Water soluble extractive	17.98
7	Foaming index	21 ml
9	pH	7.9

S.No	Chemical class	Chemical test	Ethanol
			extract
1	Alkaloids	Dragendorff's test	+
2	Naphthoquinones	Juglone test	+
2	Steroids	Salkowaski test	-
3	Carbohydrate	Molish test	+
4	Triterpenes	Vanillin-sulphuric acid test	+
5	Tannin	Ferric chloride test	+
6	Glycoside	Keller-killani test	+
7	Proteins	Biuret test	+
8	Flavonoids	Shinoda Test	+
9	Saponins	Lead acetate test	+

Table 4: Phytochemical analysis of A. indica extracts

Where + is Present and – is Absent

Figure 1: Macroscopic features of *A. indica* leaves

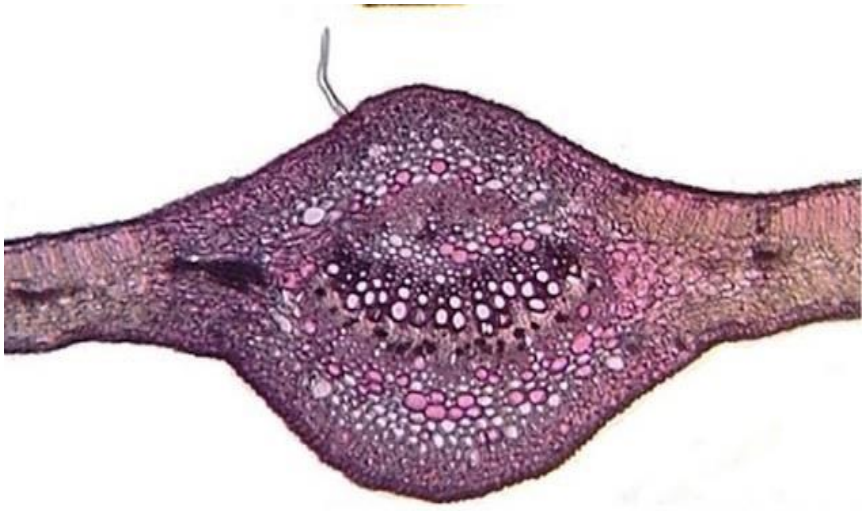
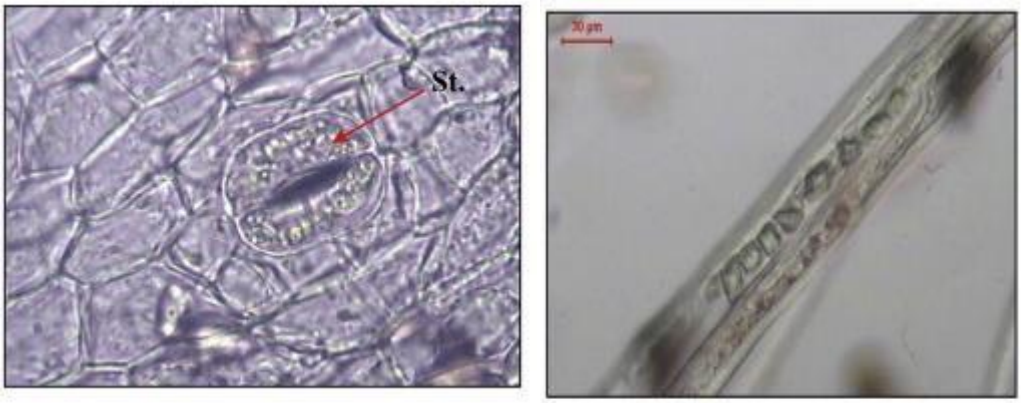


Figure 2: Transverse section of *A. indica* leaves



(a) Stomata **(b) Trachoma's**

Figure3: Powder microscopy of *A. indica* leaves

(b)

Figure 4: A. TLC of *A. indica* leaves extract

Conclusion

The Pharmacognosy-anatomical, physicochemical, fluorescence characteristics, and the preliminary phytochemical studies of the leaves part of *A. indica*have revealed the presence of phytoconstituents such as alkaloids, naphthoquinones, carbohydrate, triterpenes flavonoids, glycosides, phenols, saponins, proteins and tannins. Phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemical have been found to possess a wide range of activities.

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