

## Research Article



## Phytochemical Analysis of Different Extract of *Azadirachta indica* Leaves

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### ABSTRACT

Neem (*Azadirachta indica*) is a very useful traditional medicinal plant in the Indian sub-continent. Various plant parts of neem used to treat various ailments and diseases. Fresh matured leaves of *Azadirachta indica* were collected, dried, powered and used for further analysis. This study includes qualitative analysis of phytochemicals present in different extracts of neem leaves. The different extracts of neem leaves were prepared by using different pharmaceutical processes (Decoction, maceration, filtration etc) using distilled water and cow urine as solvents. The results of qualitative phytochemical analysis showed that different extracts of *A. indica* leaves contain phytochemicals viz. alkaloids, tannins, saponins, phytosterols. Glycoside, proteins and reducing sugar were not detected in all extract. The phenolic compounds were present in neem decoction (cow urine) and neem juice whereas not detected in neem decoction (distilled water) and aqueous extract. Thus, presence of these phytochemical suggests the pharmacological potential of the wonder tree "neem". It was concluded that in all extract of *A. indica* leaves contain pharmacologically active constituents.

**Keywords:** Neem, *Azadirachta indica*, extract, phytochemicals, cow urine.

### INTRODUCTION

India is holy land which provides shelter to the more than 7500 species of medicinal plants. The medicinal use of herbal preparations is mentioned in ancient Hindu texts like Veda and also in Ayurveda the Indian old literature on medicinal values of plants. It is a general understanding that the plants and their products are safer tools of treatment against infectious and non-infectious diseases in man and animals as compared to the synthetic's compounds. The pharmacological studies have revealed the medicinal value of plants is due to the presence of bioactive compounds. From the perspectives of discovering and designing a drug that will be safe for use in man and animals the phytochemicals present in the plants are important and should be given due consideration. Many of the plant materials used in traditional medicine are readily available in rural areas which are relatively cheaper than modern medicines. In current era quick results are expected; hence haphazard use of synthetic antimicrobial drugs is copious these days which is now resulting in multiple drug resistance and evidences of serious adverse effects are noted in various studies. Therefore, natural herbs are gaining importance in overcoming this problem as the traditional herbs are found to be more economical and having lesser side effects than synthetic drugs. Now a day's nearly 88% of the global populations turn to plant derived medicines as their first line of defence for maintaining health and combating diseases. After reviewing the vast literature on medicinal uses of plants revealed that *Azadirachta indica* is one such plant having tremendous potential of pharmacological properties.

*Azadirachta indica* commonly known as Neem is a fast-growing tropical evergreen tree found mainly in India, Africa and America. In Sanskrit, it is called 'arishtha' a word that means 'perfect, complete and imperishable and reliever of sicknesses. Neem is called by various names in India such as "Divine tree", "Wonder tree", "heal all", "Materia Medica", "Free tree of India", "Nature's drugstore", "Village Pharmacy", "Panacea for all diseases". All parts of the neem tree - leaves, flowers, seeds, fruits, roots and bark have been used traditionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. Neem leaf and its constituents have been demonstrated to exhibit immunomodulatory, antiinflammatory, antihyperglycemic, antiulcer, antimalarial, antifungal, antibacterial, antiviral, antioxidant, antimutagenic and ant carcinogenic properties <sup>4</sup>.

It is noteworthy that in India, drinking of cow urine has been practiced for thousands of years as a sort of treatment against various infectious and non-infectious diseases. In the age old year's Sushruta and Samhita the animal health care takers recommended the cow urine to cure leprosy, cardiac and kidney problems, indigestion, stomach ache, edema, etc. This kind of alternative treatment, termed as 'Panchgavya therapy' or 'Cowpathy' now a days is reported to be beneficial for dreadful diseases like cancer, AIDS and diabetes. The Panchagavya is a term used in Ayurveda to describe five important substances obtained from cow, namely, urine, dung, milk, ghee, and curd. A number of formulations mentioned in Ayurveda describe the use of Panchgavya components either alone or in combination with drugs of herbal,



animal, or mineral. The Gomutra is capable of curing blood pressure, blockage in arteries, arthritis, diabetes, heart attack, cancer, thyroid, asthma, psoriasis, eczema, prostrate, fits, AIDS, piles, migraine, ulcer, acidity, constipation, gynaecological problems, ear and nose problems and several other diseases. The Cow urine is an effective antibacterial agent against a broad spectrum of Gram-negative and Gram-positive bacteria and also against some drug-resistant bacteria. It acts as a bio-enhancer<sup>9</sup>. It can prevent, control and cure all kind of chronic diseases. It can boost the immune system, improve nervous disorder, dissolve and recover toxin which are accumulated in our body.<sup>10</sup> The objectives of this study therefore to prepare extracts of leaves of *A. indica* (Neem) in distilled water and cow urine by using different pharmaceutical process and to investigate phytochemical constituents in different extracts of neem leaves by qualitative analysis.

## MATERIALS AND METHODS

### Authentication and Collection of plant material

The leaves of *A. indica* were identified and authenticated by a Botanist, Department of Botany, Maharashtra Udaygiri Mahavidyalaya, Udgir and *A. indica* leaves were collected from campus of College of Veterinary and Animal Sciences, Udgir.

### Preparation of Extract

#### Neem Decoction (Distilled water)

Fresh *Azadirachta indica* leaves were collected and washed under running tap water and rinsed with distilled water to remove a dust and debris. The leaves were crushed in a mortar and pestle to a coarser particle size. The crushed leaves (50 gm) added with 40 ml of distilled water and boiled till the volume reduces to 20 ml. Decoction was passed through muslin cloth and allowed to cool down at room temperature. The prepared decoction was passed through muslin cloth and filtrate used for further research study.

#### Neem Decoction (Cow urine)

Fresh cow urine was passed through the muslin cloth to remove debris. Fresh *Azadirachta indica* leaves were washed under running tap water and rinsed with sterile distilled water to remove a dust and debris. The leaves were crushed in a mortar and pestle to a coarser particle size. Fresh cow urine (40 ml) and crushed neem leaves (50gm) were boiled till the volume reduce to 20 ml and the obtained decoction was passed through the muslin cloth and allowed to cool down and collected in a sterile container for further research study.

#### Neem juice

Fresh *Azadirachta indica* leaves were collected and washed under running tap water and rinsed with distilled water to remove a dust and debris. The neem leaves (50gm) were crushed in a mortar and pestle with a little quantity of distilled water (20ml) to obtain a juice. These crushed

leaves were passed through the muslin cloth and obtained filtrate was used for further research study.

### Aqueous Extract

The collected *Azadirachta indica* leaves were washed under running tap water and rinsed with sterile distilled water to remove dust and debris. The leaves were separated out from each other after wash, segregated and shed dried. The shed dried leaves were powdered using a dry mechanical grinder. The dried powder was passed through the mesh sieve to obtain the fine powder. The fifty gram of leaf powder was added with 250ml of distilled water. This conical flask was stoppered tightly and was kept at room temperature for maceration of 48 hrs and during maceration period the content of conical flask was shaken at an interval of 2 hrs. Content of conical flask was filtered through the muslin cloth. The content left in the flask was rinsed twice by taking the little quantity of distilled water and again filtered through the muslin cloth. The filtrate so obtained was once again filtered through Whatman No. 1. filter paper. The filtrate was transferred to sterilized evaporating bowl and kept under fan for evaporation of the solvent. After complete evaporation of the solvent the extract left in the bowl was taken in the airtight screw cap vials and stored in refrigerator for use as per the requirement in experimental investigations.

### Qualitative Phytochemical Analysis

All extracts of *Azadirachta indica* leaves were subjected to qualitative phytochemical analysis to identify presence of various phytochemicals viz. alkaloids, glycosides, proteins, reducing sugar, tannins, resins, sterols, phenolic compounds and saponins as per the method described by Rosenthaler (1930).<sup>11</sup>

#### 1. Test for Detection of Alkaloids:

A small amount of extract was taken in test tube and added with 5 ml of 1.5 % HCl (v/v) and then filtered. A few drops of each of the following reagents were added to the Filtrate and mixed well, appearance of turbidity or any changes in colour to the test indicates the presence of alkaloids.

##### a) Dragendorff's reagent test:

Dragendorff's reagent: It was prepared by mixing **solution -A** comprising 17 G of bismuth subnitrate and 200 G of tartaric acid added into 800 ml of distilled water and **solution- B** 160 G of potassium iodide in to the 400 ml of distilled water. Both, the solution A and B were mixed in 1: 1 proportion volume by volume. From this working standard was prepared by taking 50 ml of this solution, added with 100 G of tartaric acid to make volume up to 500 ml with distilled water.

The solvent extract (filtrate) was sprayed on a filter paper using chromatographic sprayer and was dried. The reagent was applied on above prepared filter paper using capillary tube, the development of orange to red color confirmed for the presence of alkaloid.



**b) Wagner's reagent test:**

The reagent was prepared by dissolving iodine 1.27 G and 2 G of potassium iodide in 5 ml of distilled water and diluted to 100 ml. The little amount of the above extract (filtrate) was added to this reagent, appearance of brown to flocculent precipitation revealed the presence of alkaloid.

**2. Test for Detection of Glycoside:****a) Benedict's reagent test:**

Equal quantity of both the extract and benedicts reagent was added and heated to boil for two minutes, appearance of brownish to red colour indicate presence of glycoside.

**b) Folin Wu copper reagent test:**

A little amount of extract was added to few drops of folin Wu copper reagent, the development of red colour gives positive reaction for glycoside.

**3. Test for Detection of Proteins:****a) Xanthoprotein test:**

A small amount of the extract was added with 0.5 ml of concentrated  $\text{HNO}_3$ , the appearance of white or yellow precipitate indicates the presence of proteins.

**b) Biuret test:**

Few amounts of the extracts were added to 4% sodium hydroxide solution followed by a drop of 1% copper sulphate solution, the development of violet to pink colour indicates presence of proteins.

**4. Test for Detection of Reducing Sugar:****a) Benedict's reagent test:**

The extract was added with benedicts reagent in equal amount and mixture was heated for 2 minutes, appearance of brown to red colour indicates presence of reducing sugar.

**b) Folin copper reagent test:**

Few quantities of the extract were added with few drops of folin Wu copper reagent, the development of red colour indicates presence of reducing sugar.

**5. Test for Detection of Tannins:**

A little quantity of alcohol extract taken in a test tube was warmed and filtered. The filtrate was used to carry out the tests.

**a) Lead acetate test:**

Few drops of 5% lead acetate solution were added to the filtrate, formation of precipitation indicates the presence of tannins.

**b) Ferric chloride test:**

Few drops of ferric chloride were added to the little amount of the filtrate, development of green color revealed presence of tannins.

**6. Test for detection of phytosterols:****a) Salkowski reaction:**

A small amount of extract was added with 2 ml of concentrated  $\text{H}_2\text{SO}_4$  and was shaken for few minutes and mixed well, the development of red or brown colour indicates the presence of sterols.

**7. Test for detection of phenolic compounds:**

A small amount of extract was treated with 2 ml of ferric chloride solution and shaken for few minutes. The appearance of pale brown colour to the test indicates presence of phenolic compounds.

**8. Test for Saponins:****a) Foam test:**

A small amount of extract was treated with 2ml of sodium bi-carbonate and added with distilled water, the mixture shaken vigorously. The development of froth to the test indicates presence of saponins.

**RESULTS AND DISCUSSION**

The results of qualitative phytochemical analysis of *Azadirachta indica* leaves extracts is summarised in table 1.

The results showed that alkaloids, tannins, phytosterols and saponins were detected in all extracts of *A. indica* leaves. Phenolic compounds were detected in Neem leaves decoction (Cow urine) and fresh juice of Neem leaves (Distilled water), whereas not detected in Neem decoction (Distilled water) and aqueous extract of *A. indica*. Glycosides, proteins and reducing sugars were not detected in all extracts of *A. indica*. The medicinal values of the secondary metabolites are due to the presence of chemical substances that produced by the plant extracts against particular organism produce a definite physiological action on the body<sup>12</sup>.

The phytochemicals exhibit various pharmacological and biochemical action when ingested by animals. The plant bioactivity depends on chemical compounds which may inhibit insect feeding. Agro ecosystems are an important system for secondary plant metabolites and their degradation products. Toxic effects to insects/pests are produced by the compounds viz. terpenoids and steroids, phenols, coumarins, flavonoids, tannins, alkaloids, and cyanogenic glycosides.<sup>13</sup>

Alkaloids are organic nitrogenous substances (alkaline in nature) having remarkable physiologic and pharmacologic properties like stimulant, spasmolytic, vasodilator, anti-asthmatic, anti-arrhythmic etc.<sup>14</sup> The presence of alkaloids represents the possibility of some biological activity of the extracts of *A. indica* such as anti-cholinergic, anti-tumour, anti-hypertensive, cough expectorant, anaesthetic, analgesic, muscle relaxant, anti-pyretic, anti-malarial<sup>15</sup>. The most important of these substances include alkaloids, tannins for cell growth, replacement and body building. Plant extracts are potential sources of novel therapies.<sup>12</sup>



**Table 1:** Phytochemical analysis of different extracts of *Azadirachta indica* leaves.

Sr No	Phytochemicals (Active Constituents)	Tests	Neem Decoction (Distilled water)	Neem Decoction (Cow Urine)	Neem Juice (Distilled water)	Aqueous Extract of Neem
1.	Alkaloids	Dragendroff's	+ ve	+ ve	+ ve	+ ve
		Wagner's	+ ve	+ ve	+ ve	+ ve
2.	Glycosides	Benedict's	-ve	-ve	-ve	-ve
		Folin Wu's	-ve	-ve	-ve	-ve
3.	Tannins	Lead acetate	+ve	+ve	+ve	+ ve
		Ferric chloride	+ ve	+ ve	+ ve	+ ve
4.	Proteins	Xanthoprotein	- ve	- ve	- ve	-ve
		Biuret	-ve	-ve	-ve	-ve
5.	Reducing Sugars	Benedict's	- ve	- ve	- ve	-ve
		Folin Wu's	- ve	- ve	- ve	-ve
6..	Phytosterols	Salkowski	+ ve	+ ve	+ ve	+ ve
7.	Phenolic Compounds	FeCl <sub>3</sub> Solution	-ve	+ ve	+ ve	-ve
8.	Saponins	Foam	+ ve	+ ve	+ ve	+ ve

**Note:** '+ve' indicate Positive, '-ve' indicate Negative.

Saponins consist of polycyclic aglycones attached to one or more sugar side chains. Saponins have many health benefits such as the beneficial effects on blood cholesterol levels, cancer, bone health and stimulation of the immune system. Saponins show anti-fungal, antibacterial and anti-protozoal effects.<sup>14</sup> Aqueous leaf extract of *A. indica* showed the presence of alkaloids, saponins which have nematocidal properties.<sup>16</sup> Phytochemicals like alkaloids, saponins and Tannins are known to induce analgesic mechanisms and minor anti-oxidant activity.<sup>2</sup>

The presence of tannins represents the possibility of some biological activity of the extracts of *A. indica* such as anti-diarrheal, haemostatic, anti-hemorrhoidal, anti-inflammatory, astringent, and anti-infective. It can be used for immediate relief of sore throats, diarrhoea, dysentery, haemorrhaging, fatigue, skin ulcers and as a cicatrizing on gangrenous wounds. Tannins can also be used against poisons. It also possesses antioxidant effects. All these effects may be due to the presence of tannins in the extract of *A. indica* because tannins are previously reported to show such effects.<sup>15</sup> Presence and absence of the phytochemical constituents depend on the test applied for the qualitative detection of secondary metabolites.

Phenolic compounds in *A. indica* leaves extracts have been reported to be associated with anti-oxidative action which provides protection against free radicals that damage cells and tissues.<sup>14</sup>

## CONCLUSION

From the above study, it can be concluded that alkaloids, tannins, phytosterols and saponins were present in all extract of *A. indica* leaves. Phenolic compound was only detected in Neem leaves Decoction (cow urine) and fresh juice of Neem leaves (distilled water) whereas not detected in Neem leaves Decoction (Distilled water) and aqueous

extract of *A. indica*. Thus, different Neem leaves extract could be considered responsible for conferring different pharmacological properties viz. Analgesic, muscle-relaxant, spasmolytic, anti-asthmatic, anti-arrhythmic, anti-cholinergic, anti-tumour, anti-diarrhoeal, anti-inflammatory, astringent, anti-viral, antifungal, antibacterial, anti-protozoal etc. Cow's urine therapy treatment is most effective natural remedy and safest method of treatment without any side effects.

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