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# A NOVEL STUDIES ON TREATMENT OF HERPES SIMPLEX INFECTION ON SKIN BY NEEM SEED OIL

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## ABSTRACT

*Azadirachta indica* is a fast growing evergreen popular tree found commonly in India, Africa and America. Neem is a Native and Traditional tree in various areas. Neem oil extraction from Neem seeds (*Azadirachta indica*) with n-hexane and ethanol. Used as complementary medicine for like phytotherapy. The potential effects of the extract are used to attributed cellular and molecular mechanisms, detoxification, DNA repair, anti-inflammatory, anti-angiogenic, and anti-metastatic activities. Neem seed can be used for various Skin infections, Dentistry, Anti-diabetic, Anti-microbial activities. Neem seeds are mainly used in dried condition known as Kernels that helps in easy extraction of oil.

Keyword : Neem, *Azadirachta Indica*, Neem oil, Neem seed, Extract.

## INTRODUCTION

Neem tree, which is also known as *Azadirachta indica*, is one of the best known trees in India, which is known for its medicinal properties. The Neem tree, is primarily cultivated in the southern regions of Asia and Africa, where it has been seen used through many ages, in medical folklore. It grows in much of Southeast Asia and West Africa; a few trees have recently been planted in the Carribbean and several Central American countries, including Mexico.(1) The people of India have long revered the neem tree; for centuries, millions have cleaned their teeth with neem twigs, smiered skin disorders with neem-leaf juice, taken neem tea as a tonic, and placed neem leaves in their beds, books, cupboard. The number of benefits of neem is listed in ancient documents like ‘Charak-Samhita’ and ‘Susruta-Samhita’. It is commonly called ‘Indian Lilic’ or ‘Margosa’, belongs to the family Meliaceae, subfamily Meloideae and tribe Melieae. Neem is the most versatile, multifarious trees of tropics, with immense potential.

It possesses maximum useful non-wood products (leaves, bark, flowers, fruits, seed, gum, oil and neem cake) than any other tree species known to have antiallergenic, antidermatic, antifeedent, antifungal, antiinflammatory, antipyorrhoeic, antiscabic, cardiac, diuretic, insecticidal, larvicidal, nematocidal, spermicidal and other biological activities. Because of these activities neem has found enormous applications making it a green treasure. Neem has become important in the global context today because it offers answers to the major concerns facing mankind. Neem for birds, beneficial insects and earthworms has been approved by the US Environmental Protection Agency for use on food crops.

We should note that various parts of the Neem tree, including the leaves, bark, fruit, flowers, oil, and gum are associated with the mentioned medical folklore in the treatment of certain medical conditions such as cancer, hypertension, heart diseases, and diabetes. The potential effects that are seen when using these extracts can certainly be attributed cellular and molecular mechanisms, these mechanisms include free radical scavenging, detoxification, DNA repair, cell cycle alteration, programmed cell death mitigation and autophagy, immune surveillance, anti-inflammatory, anti-angiogenic, and anti-metastatic activities and the ability to modulate of various signaling pathways.

Extraction of oil has been of great interest worldwide and this has been as a result of the constant increase in the world population. The Neem oil produced cannot cater for all need of the population which includes domestics and industrial uses. Neem oil extract, which is the fatty acid-extract of Neem tree seeds, is the most widely used product of the Neem tree. Neem seeds contain about 25 - 45% oil and provide the major source of Neem chemicals such as Azadirachtin, Nimbin.

## **ADVANTAGES AND DISADVANTAGES**

### **Advantages:**

1. It can boost Dental and oral health.
2. It improves skin care.
3. Cost effective due to lesser steps.
4. It can promote hair health.
5. It can help in fight with oxidative stress which inturn promote the liver and kidney health.

### **Disadvantages:**

1. Special grade excipients are required.
2. Tablets produced are softer than wet granulation leading to difficulty in processing techniques afterwards.
3. This method avoids when colourants are used as the dosage form appears mottled.

## **METHODS OF PREPARATION**

Mainly the preparation of NSO tablets from Neem seed oil (NSO) includes various steps. Firstly the oil is extracted from Neem seed kernels. Solvent extraction process is used for the extraction of pure oil. This Tablets are prepared by using Direct Compression process for better results of formulation of tablets.

## A) Extraction of Oil from Neem Seeds

- a. Mechanical pressing
- b. Steam distillation extraction
- c. Solvent extraction
- d. Super critical extraction
- e. Aqueous extraction

### a. Mechanical Press Method:

This method is one of methods of processing oil. Seeds are placed in a tub or container and a form of press or screw is used to squeeze the seeds until the oil is pressed out. Mechanical extraction of Neem seeds performs using hydraulic pressing equipment. Untreated seed particles to be pressed with various pressures to determine the optimum pressure. Pressure was started at 138 Bar as the oil started to flow out of the seedbed, and stopped at 412 bars since the oil yield relatively constant at the pressure above 413 bars. Mechanical extraction was performed for 25 minutes when the oil has stopped flowing out. Oil yield measurement was conducted using mass balance. Neem seeds kernels are placed in to a vessel and either a screw or some of form of press is used to squeeze the kernels under pressure until the oil is pressed out and collected. The neem oil is obtained by pressing it mechanically and collected in a drum. Thus filtration is done to remove the various unwanted particles left in the extracted oil in order to obtain pure neem oil.

### b. Steam Distillation Extraction:

This method uses steam and high pressure to extract the Neem oil. The kernels are heated with steam to increase the Neem oil flow, then squeeze under high pressure. Most of the Neem oil is extracted from the kernels. This procedure makes the extraction process easier. As for the steam distillation process, the dried Neem seeds are put into the steam boiler. Then they get swollen by steaming, thus Neem oil in squeezing becomes very easy. This steaming process is accompanied by increasing pressure in the boiler which drives the oil out of Neem seeds.

Steam distillation is a separation method which is used for separating a mixture of components which are heat sensitive by using steam. The principle is based on variation in the boiling point of reducing the partial pressure of volatile components. Steam distillation has important application in petroleum refineries, extraction of volatile compounds of plants which were used in perfumery and flavoring industries. Catnip is extracted using steam distillation. The steam distillate content has two parts, essential oil, and distillation water. The steam distillate is subjected to solvent extraction where the secondary essential oil is extracted.

The oil is extracted from the entire plant using a distillation element consisting of a distillation tank, a condenser, and a separator. Freshly harvested grass as such or after cutting into little pieces is loaded into the distillation tank. After closing the lid tightly, steam is entering into the tank. Steam and the oil vapor condense into liquid in condenser & are collected in the separator.

### c. Solvent extraction:

The oil can be obtained by pressing or crushing of the seed kernels both through cold pressing and through a process incorporating temperature controls 40-50 °C. Neem seed oil can be obtained by solvent extraction of the neem seed, fruit, oil, cake or kernel. A large industry in india extracts this oil

remaining in the seed cake using hexane. This solvent extraction oil is of lower quality as compared to cold pressed oil and is generally used for soap manufacturing. Neem cake is a by-product obtained in the solvent extraction method for Neem oil.

40g of Neem seeds were weighed and put into the thimble of the Soxhlet extractor. 300ml of the solvent or ethanol was measured with a measuring cylinder and poured into the still pot of the Soxhlet extractor, the apparatus was then coupled and the condenser unit was connected to an overhead water tank to cool rising solvent vapor. The heat source was a Bunsen burner operating at a temperature of 68°C. The solvent evaporated during the distillation path, thimble, and the expansion adapter after which it condensed at the condenser unit of the Soxhlet extractor. At this position, the condensed vapor returned to the thimble as liquid droplets and got in contact with the sample therein. It then broke the sample membranes to release the Neem oil content which accumulated with the solvent at the siphon (or reflux arm) of the Soxhlet extractor. When the solvent in the thimble rose to the point of the siphon top, the entire content of the thimble and siphon was emptied back into the still pot of the Soxhlet extractor. The process was repeated severally for about nine refluxes in three hours after which the extraction process was completed. The temperature was regulated using a thermometer.

#### d. **Super Critical Extraction:**

The supercritical fluid extraction process is the most effective and efficient way to extract valuable constituent botanicals. This is the process of separating one component or the extractant from another or the matrix using supercritical fluids that is carbon dioxide as the extracting solvent. Carbon dioxide solvent is the king of extraction solvents for botanicals. Extraction conditions for supercritical CO<sub>2</sub> are above the critical temperature of 31°C and pressure of 74 bar. Supercritical fluids are extremely compressed gases, which have combined properties of gases and liquids in an intriguing manner. Supercritical fluids can lead to reactions, which are difficult or impossible to achieve in conventional solvents. It is a fast procedure completed in 10 to 60 minutes. A supercritical extraction fluid can be separated from analyte by simply releasing pressure, leaving almost no trace and yields a pure residue.

#### e. **Aqueous Extraction:**

The aqueous extraction is the simplest method and most used is the extraction of water. It consists of crushing or grinding the seed or Neem leaves, put in water, strain into the thin fabric and collect the Neem extract. This Neem oil extract can be used in a spray for the control of pests without modification.

### **B) Conversation of Oil to Powder :**

Measure the oil upto 1.5ml, weigh the required amount of starch diluent that will not effect the activity of the oil. Sodium chloride is used as Preservative. Add all the three ingredients into the motor and pistle mix thoroughly to avoid lumps. Dry this mixture in the hot air oven at 50°C for 30 minutes.

#### **C) Formulation of Tablet:**

Granulation is the process in which primary powder particles are made to adhere to form larger, multi particle aggregates called granules.

- a. Wet Granulation
- b. Dry Granulation
- c. Direct compression

**a. Wet Granulation :**

Wet granulation is a widely used method for the production of compressed tablet. It is essentially a process of size enlargement involving several steps and the use of an adhesive substance known as binder. Steps involved in Wet Granulation are Weighing, milling and mixing of the APIs with powdered excipients (excluding the lubricant), Preparation of binder solution, Mixing of binder solution with powders to form a damp mass, Screening the dampened powder into pellets or granules (wet screening) using 6- to 12-mesh screen, Drying of moist granules, Sizing the granulation by dry screening using 14- to 20-mesh screen, Mixing of the dried granules with lubricant and disintegrants and finally Compression of granules into tablets.

**b. Dry Granulation:**

The formation of granules by compacting powder mixtures into large pieces or compacts which are subsequently broken down or sized into granules that are possible for granulation method which, however, is not widely used in the manufacture of tablets. This method is used when tablet excipients have sufficient inherent binding properties. Compaction for the dry granulation process is generally achieved either by slugging or roller compaction. Steps involved are Weighing and Milling of formulation ingredients (drug substance and excipients), Mixing of milled powders, Compression of mixed powders into slugs, Milling and sieving of slugs, Mixing with disintegrant and lubricant and then Compression into tablets.

**c. Direct Comparison :**

Direct compression involves, direct compression of powdered materials into tablets without modifying the physical nature of the materials. Direct compression avoids many of the problems associated with wet and dry granulations. Steps involved in direct compression are Milling of therapeutic agent and excipients, Mixing of milled powders, disintegrants and lubricants and finally Compression into tablets.

**USES**

Neem seed oil is mainly used for the skin infection, itching, skin ulcers, burning sensation and leprosy where the Neem tablets are used for the treatment of Anti-inflammatory, Anti-diabetic, Anti-microbial, Dentistry, wound healing and Growth promoting effect.

**EVALUATION**

Evaluation methods for tablets includes Weight Variation, Thickness, Hardness, Friability, Drug Content Uniformity, Disintegration Test, *Invitro* Dissolution Studies, *Invivo* Dissolution Studies and FTIR.

**Weight variation:**

The weight variation test would be a satisfactory method for determining drug content uniformity of drug distribution. In practice this test is performed by taking 20 tablets, from a batch. 20 tablets are weighed at a time and the average weight is taken. Then the tablet is weighed individually.

130 mg or less.	10
More than 130 mg through 324 mg.	7.5
More than 324 mg.	5

### **Thickness:**

The thickness of individual tablets is measured with a micrometer, which gives us information about the variation between tablets. Tablet thickness should be within a  $\pm 5\%$  variation of a standard value. Any variation in thickness within a particular lot of tablets or between manufacturer's lots should not be clear to the unaided eye for consumer acceptance of the product. In addition, thickness should be controlled to smooth the progress of packaging.

### **Hardness :**

Tablet hardness is the force necessary to break the tablet diametrically. The tablets must be hard enough to withstand mechanical stress during packaging, shipment, and handling by the consumer. To test the hardness of the tablet Monsanto tester, Strong-cobb tester, the Pfizer tester, the Erweka tester, the Schleuniger tester are used.

### **Friability:**

Tablet friability represents the tendency of a tablet to shed powder or break into smaller pieces under mechanical stress, such as falling from a fixed distance. It is a function of the fragility of the compressed powder blend, tablet shape, cohesion, and hardness. Low tablet friability is desired to ensure its physical integrity during packaging, shipment, and handling.

### **Drug content Uniformity:**

All tablets must be demonstrated to contain the labeled active ingredient and there should be tablet-to-tablet uniformity in drug content. This is usually tested by an analytical method for drug potency (such as highperformance liquid chromatography) in a several individual tablets.

### **Disintegration:**

Disintegration of tablets is evaluated to ensure that the tablet dissolves or breaks apart into smaller particles or granules on contact with water under agitation. This allows the DS to dissolve from its primary particles, being fully available for dissolution and absorption from the GI tract. Tablet disintegration is evaluated in a standardized apparatus that subjects six tablets to a defined mechanical stress in individual reciprocating cylinders in a suitable aqueous medium at  $37^{\circ}\text{C}$ , to reflect the conditions on oral ingestion. The time it takes for the last of six tablets to disintegrate into smaller particles and disappear from the reciprocating cylinders is called disintegration time. The disintegration media required varies depending on the type of tablets to be tested. The disintegration time is generally not more than 15 min for IR tablets. The disintegration test is used as a control for tablets intended to be administered by mouth, but not for the tablets intended to be chewable and SR.

**Invitro Dissolution Studies:**

Stated volume of the dissolution medium, free from dissolved air was introduced into the vessel of the apparatus. The dissolution medium was warmed between 36.5° and 37.5° . The tablet was allowed to sink to the bottom of the vessel prior to the rotation of the paddle. Wire helix was used to keep the tablet horizontal to avoid floating at the bottom of the vessel. Air bubbles were removed from the surface of the tablet. A sample from the surface of the dissolution medium was withdrawn, analysed for absorbance as directed in the standard monograph using spectrophotometer. The whole operation was repeated for five times and the amount of dissolved active ingredient of the tablet in the solution as a percentage of the stated amount was calculated.

**Invivo Dissolution Studies:**

According to Ege University, Faculty of Medicine, Ethical Committee. Tablets with 4 mm diameter were administered orally via a silicone rubber gastric intubation tube to male albino rabbits with average weight 2.5 kg which were housed individually in standard cages in a room with air, humidity, and temperature control. The rabbit's mouth was opened, and a wooden rod was inserted between the jaws. A gastric tube was centrally placed to the hole. Tablets were set on the tip of a gastric intubation tube, and administered into the stomach of rabbits. After receiving the oral dose, 5 ml of water was administered to facilitate the accession of tablets. Rabbits were fasted overnight but had free access to water; 3 mL blood samples were withdrawn from a heparinized branch (G22, G24), placed in the marginal vein, at each of the pre-determined times after administration: 10, 20, 30, 60, 90, 120, 180, 240, 300, 360, 420, 480, 600, and 720 min. Samples were transferred to 4 mL blood tubes and centrifuged at 3500 rpm for 10 min by IEC PR-6000 Centrifuge (Damon, USA). Separated supernatant was taken into Eppendorf tubes (Damon, Massachusetts, USA) and stored at -20°C until analyzed.

**FTIR studies:**

10 Tablets were triturated after taking their average weight. The tablet powder equivalent to 1 tablet was transferred to the volumetric flask and dissolved in chloroform. The resulting solution (100µg/ml) was sonicated for 10 min and supernatant was filtered through whatman filter paper no. 41. Filtrate was evaporated and from the residue obtained 1 mg was accurately weighed, made up to 100 mg with dried KBr and triturated well. Then the dilution is further performed from this stock mixture to prepare the pellet of desired concentration. Thereafter the concentration of the sample and Absorbance are represented in graph.

**APPLICATIONS**

Applications of NSO tablets are Anti-inflammatory, Skin infection, Anti-diabetic and Dentistry.

**CONCLUSION**

It has been concluded that the better results are observed in the treatment of skin infections like herpes simplex infection by the Neem tablets prepared by Neem seed oil. Most of the people are suffering from skin infections. So, by this novel drug delivery shows better impact and treatment for skin infections.

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