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EXTRACTION OF NEEM OIL WITH DIFFERENT METHODS AND APPLICATIONS IN DIFFERENT FIELDS

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Abstract: *The current review gives an insight into the chemistry of neem oil extraction. It reviews functional and chemical properties of the phytochemical, advanced delivery system, preparation techniques, various applications, and risk assessment on human health and environments along with current and future prospects of Neem oil. Neem oil is a natural antimicrobial, anti-oxidant, and an effective pesticide and insecticide that has been utilized in storage of food grains and in organic agricultural farming for several decades. It has been used as a therapeutic vaccine in the treatment of skin conditions analogous to eczema, furunculosis, arsenical dermatitis, scabies, and seborrheic dermatitis. The neem plant-based insecticides have been the most widely used bio-pesticides among the various herbs because they contain a variety of limonoids that not only offer a long-lasting pest control mechanism but also guard against plant disease resistance to various synthetic insecticide and pesticide, boosting crop productivity and yield*.

Keywords – Neem oil, Extraction, Natural, Treatment**.**

1. INTRODUCTION

Neem oil is a vegetable oil obtained from the fruits and seeds of the Neem tree (Azadirachta indica). It has been used for centuries in traditional Indian medicine and is now used in a wide range of applications due to its many beneficial properties. In recent years, extraction of Neem oil has become increasingly common, and the methods used to extract the oil vary. Neem oil has been used for centuries in traditional Indian medicine for its medicinal properties. It is known to have anti-inflammatory, antimicrobial, and antifungal properties, which makes it beneficial in treating a variety of skin and hair conditions. It is also known to be an effective insect repellent, which is why it is often used in organic farming. In addition, its taste and smell can be used to naturally flavor food, which has become increasingly popular in recent years. This research paper aims to investigate the extraction of neem oil with different methods, as well as the potential applications of the extracted oil in different fields. Neem oil is a vegetable oil obtained from the leaves, bark and seed kernels of the neem tree. The review will evaluate the different extraction methods and discuss their efficacy, cost-effectiveness, and safety. Furthermore, the paper will assess the potential uses of neem oil in various fields and discuss the benefits and risks associated with them.

The extraction of Neem oil is a complex process and there are several methods used to extract the oil from the seeds. These include mechanical pressing, which is the most common method, solvent extraction, and steam pressure extraction. Each method has its own advantages and disadvantages, and it is important to choose the right method for the desired application.

2. RESEARCH METHODOLOGY

There are many methods in use nowadays for extraction of neem oil. The four main methods of extraction are:

1. Mechanical process
2. Steam Pressure Extraction
3. Solvent Extraction
4. Supercritical Extraction

These methods are used for extraction of neem oil [1]. Kernels collection: Kernels are also known as seed. These are extracted from the ground very neatly and cleanly so we get the clean seed. Good quality of neem kernels are required to get the neem products [1].

1. Mechanical Process: In this process we take the neem seeds and keep them in a tub or container. After that they are pressed with a big screw. For that we use hydraulic pressing equipment. To get constant flow of oil from the seed we use different pressures to reach that flow. It starts at 138 bars as the oil starts to flow from seeds. It stops at 412 bars as oil is constantly flowing at 413 bars above. This process is done until the flowing of oil is stopped. This we get at 25 minutes. This oil is collected in big drums or containers. Then it goes for a further purification process so that the unwanted particles come out and are separated from it. After purification we get the pure neem oil. Thus the oil is measured by the mass transfer operation [1]. Here we can also do the pretreatment of neem seeds like heating the seeds at different temperature to know about changes in yield of neem oil [13].
2. Steam Pressure extraction: In this method we use steam and high pressure. The seeds are heated with steam to increase the oil flow. After that at high pressure they get squeezed. Due to steam the seeds get swollen and the process gets easier to extract oil. If we steam the seeds at high pressure in the boiler the oil flows out from the seeds. This reduces the squeezing process. Oil gets out of seeds because of water oil emulsion. After steaming process in some industries the seeds are taken out and sent for further extraction so yield of neem oil will increase [1].
3. Solvent Extraction: Extraction of neem oil via solvent extraction. In this process we use three different solvents n-Hexane, ethanol [13][15-17] and methanol [12]. The apparatus used for this process is Soxhlet apparatus. Here we may vary several parameters to get good quality and yield of oil:
    a. We can also use other solvents like isopropanol, acetone. We can use it directly as pure solvent or with water and solvent [14].
    b. In the first case we heat the solvent (n-Hexane) at constant temperature and also the constant seed size at three different time intervals to find difference in yield [12].
    c. In the second case we keep the seed size constant but change the temperature of the solvent (n-hexane) to get a difference in yield [12].
    d. In the third case we change the volume of solvent (mixture of n-hexane and ethanol in the ratio of 40:60) at constant temperature at 3 different time intervals to observe the difference in yield [12].
    e. in the fourth case we use the solvent i.e. methanol keeping constant temperature and seed size. But in this case, we did not get the oil in 1hr and we had to keep it for 3hr process [12].
    f. In the fifth case we use solvent (Ethanol) keeping constant temperature and seed size at 3 different time intervals to observe the yield [12].
    g. In the last case we use a solvent mixture (Ethanol and n-hexane in the ratio of 40:60) at constant temperature and seed size but at different time intervals [12].

We can also use other solvents like isopropanol, acetone. We can use it directly as pure solvent or with water and solvent [14].

2.1 COMPARISON OF DIFFERENT RAW MATERIALS LIKE BARK, SEEDS AND LEAVES

In effect, neem leaves, seeds, and bark can all be used to extract oil, each needing a different or similar procedure as discussed above [1][13][15][16]. The plant oils can be broadly divided into two categories: fixed oils and essential oils. The essential oils are volatile and are typically obtained from the plant components other than the seeds. The majority of "fatty oils," which are fixed oils, come from seeds [22]. The methods used to characterize the oil extract are briefly highlighted in the following chapter [22]. i) Oil yield ii) Moisture content iii) Density iv) Acidic value v) Iodine value

The results for the identified oil are presented in Table 1.

The oil that is derived from each raw material also has a variety of applications. The following list includes a few of them:

(i) Neem's (Azadirachta indica) ability to fight oral cancer. Neem has untapped potential as a therapeutic vaccine in the future, according to the immunomodulatory properties of its numerous components. It is advocated that larger clinical trials be conducted to fully realize the great potential of neem and its ingredients for treating and preventing cancer [22][23][24].

Property/ Sample	Neem Seed	Neem Leaves	Neem Bark
Oil yield	34.85%	37.73%	27.70%
Moisture content	41.0%	57.6%	34.2%
Density	1089 kg/m3	1108 kg/m3	1029 kg/m3
Acidic value	12.34	10.56	13.46
Iodine value	7.61	-	-

Table.1 Characterization of oil extracts

(ii) For malaria prevention. Neem oil formulations' capacity to kill An. gambiae larvae at relatively low concentrations suggest the possibility of their application as synthetic insecticide alternatives in the management of malaria vectors. Better protection, similar to that attained with some commercial synthetic insect repellants, would be gained with repeated applications over time.

(iii) For Water Treatment. When combined Azadirachta indica with Moringa oleifera leaf extract it can significantly improve the physiological (pH, turbidity, total dissolved solids) and chemical (fluoride concentration) characteristics of ground water at a dose comparable to that of the individual extracts.

(iv) Used as a bio-pesticide. Neem plant-based insecticides have been the most widely used bio-pesticides among the various herbs because they contain a variety of limonoids that not only offer a long-lasting pest control mechanism but also guard against plant disease resistance to various synthetic insecticides, boosting crop productivity and yield.

3. Applications of neem oil

As we know neem tree has been present in Indian subcontinent for centuries and ancient literature of India also recognizes the importance of the neem oil when we read various literatures related to ayurvedic systems. Just like the ancient applications below are the day to day applications of neem oil extract in various industries.

A) Pharmaceutical Industry

In the pharmaceutical industry there are numerous applications of neem oil used for its medicinal purposes. As a anti inflammatory property *nimbidin* is triterpenoid isolated from neem. It is a mixture of tetranor triterpenes. It is a very important constituent from the neem seed oil. Nimbidin is used to cure certain inflammatory conditions. A study was done on *nimbidin* that showed that *nimbidin* helps to control the mechanisms related to macrophages and neutrophils showing relevance to its anti- inflammatory mechanism [2]. In anti- viral properties there are various anti-viral properties of neem oil extract. It prevents the growth of the dengue virus [5]. First reports that were found regarding antiviral activities of neem oil was in 2007 by Indian journal of medical research [6]. In that research they found out a compound named nimba flavone was effective against influenza virus and especially against Hiv [4]. In diabetes it is due to the bitterness of neem oil it is used effectively against the diabetes by studying the hypoglycemic effect of diabetes due to this property neem extract is used in several diabetic medicines [7] talking about cancer. It is the most important application of neem oil. The study of neem extract showed that the 7-12-dimethyl-benz-[o]-anthracene compound found in neem oil showed antiproliferative properties [8]. Also neem oil component showed increased apoptotic effect against the cancer cells that are mostly found in the human beings including prostate[10], cervical [11], leukemia[9]. Also, the study found out that neem extract helped disrupting the cell migration.

B) Food Industry

Neem oil is completely natural, bioactive plant extract obtained primarily from the neem seeds. It has excellent antimicrobial, antioxidant, pesticidal and insecticidal properties making it an attractive alternative to synthetic chemicals that are used in application of food preservation, packaging and storage. Neem oil and its components (e.g., azadirachtin) have been used in storage of food grains for many years. Photosensitivity, rapid degradation, and impact on sensory qualities of food pose major challenges against applications in food preservation and the packaging. Nanoemulsions of neem oil are gaining research attention in various fields including food technology and agriculture sectors, as they are more stable, efficient, and possess improved functionalities and properties. Neem oil and its nanoemulsion has also been used in chitosan, starch, or pectin based active packaging of foods including fruits and vegetables and other consumable products. Various studies have reported that careful use of neem oil and its components in food preservation, packaging, and coatings are for good human health and also are eco-friendly. The current review provides an insight into the chemistry, functional and chemical properties of neem oil, advanced delivery system, nanoemulsion preparation techniques, various applications of neem oil and nanoemulsion for food preservation and packaging and also risk assessment on human health and environments along with current and future prospects.

C) Nano emulsion For food preservation and packaging-

Neem has been regarded as the powerful natural preservative that has many applications in the food sector. Using neem oil or neem leaves or other neem derived active components for protection of stored food grains as preservatives, pesticides, and insecticides are age-old practices followed by farmers and growers [29] and it has been reported that 20 mL of neem oil sprayed on 1 kg pulses effectively repelled insects such as weevils, red flour beetles, fig moth, long-headed flour beetle, etc. during storage of the food grains [29]. Neem seed powder (4% by wt.) and leaf powder (5% by wt.) were effective natural pesticides for storage of maize for up to 5 months [30]. In the same study, commercial neem oil preparation such as nimbecidine (1–2% neem oil) showed even better pesticidal activities, and protected the stored maize for up to 9 months [30]. More recently, NeemPro, a commercially available pesticide containing 0.03 g/kg of Azadirachtin, was used at a concentration of 6 g/kg to store maize grains, and the treatment killed all the maize weevils without affecting their germination during 4 months of storage [28]. Neem oil is used in food preservation against microbial spoilage due to its active antibacterial and antifungal properties. The main active constituents are azadirachtin and salanin in neem oil and neem cake, respectively[31][32], which are antibacterial and antifungal in nature, and can be effectively used in food preservation and storage, either on its own or in a mixture with a variety of hurdles. Fruits and vegetables are susceptible to rapid microbial spoilage, and their postharvest losses are of serious concern, as almost 25–30% of the total production are wasted during postharvest handling. Although, neem is enlisted by the US Environmental Protection Agency as a food crop, and neem oil is considered to be a GRAS substance [27], use of neem oil directly on food surfaces or in food is avoided due to bitterness of the plant

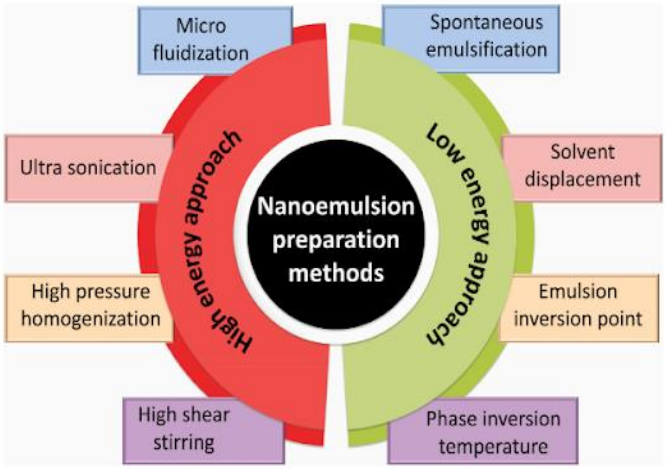

Fig.1.Various preparation method for nanoemulsions.

extract[68].The terpenoids such as nimbidin, nimbinin, nimbin and nimbidin are primarily responsible for bitterness in neem oil. Extraction of seeds using a polar solvent [34] or by adsorbent based strategies such as solid phase extraction (SPE) [33] removes these water-soluble,bitter terpenoids, followed by a hexane extraction that renders the oil tasteless, odorless, pale yellow in color. This debittered neem oil can be effective antimicrobial and/or antioxidant, and a natural food preservative. Dried neem leaves and/or 1bark have also been used as a natural preservative for food grains storage (e.g., rice) for centuries in South East Asia, including India [17]. Today, neem oil is used at 100 µL per 10 g concentration in retail meat inoculated with 4 log CFU mL− 1 of common meat spoiling microorganisms such as Carnobacterium maltaromaticum, Brochothrix thermosphacta, E. coli, Pseu- domonas fluorescens, Lactobacillus curvatus and L. sakei [26]. Almost all spoiling microorganisms were undetectable and non-identical in the treated meat after two days of refrigerated (10˚C) storage, whereas the spoiling micro-organisms were detected in untreated meat. The antimicrobial activity of neem oil in the meat was comparable with that of the commonly used antibiotic ciprofloxacin [26]. However, direct applications of neem oil on fruits and vegetables or in milk and juice are rare.

D) Biopolymer-based sustainable food packaging

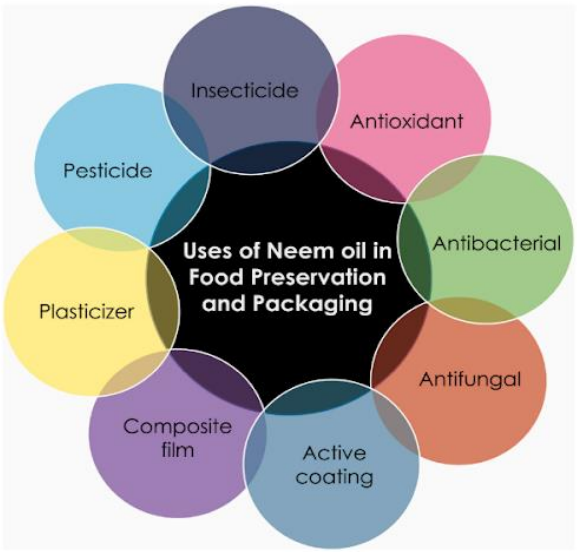

Fig.4.Uses of neem oil in food industry

Food packaging and preservation play an important role in protecting food products from the external environment and microbial activities, and their primary goal is to satisfy both industrial and consumer requirements by ensuring food safety and minimizing environmental effects [36,37]. Active packaging containing natural antimicrobials and antioxidants are novel technologies to elongate postharvest shelf-life of perishable food items, and to maintain or improve the quality and safety of prepared food products [38,39].

Innovative and sustainable food packaging such as biodegradable, recyclable, non-toxic, and active films and coatings can be developed using neem oil as a bioactive component [40,41]. As opposed to synthetic wax-based films or coating, the biopolymer-based coating (e.g., chitosan, starch, pectin, etc.) with added neem oil is totally edible and harmless to human health and environment [42]. The use of plant derived extract is an innovative way for sustainable antimicrobial food packaging. Paper and paperboard-based packaging are easily penetrable by insects and/or pests, and neem oil has been traditionally used in such packaging materials as insecticide, pesticide, and as antimicrobial agent [43]. [41] developed chitosan-based packaging films with added neem oil and zinc oxide nanoparticles, and found that the composite films had stronger tensile strength, elasticity, transparency and decreased barrier properties and solubility due to the presence of neem oil. Also, the film showed good inhibition effects against *Escherichia coli.* Improved tensile strength, hydrophobicity and antimicrobial activities rendering them potential choices of sustainable and efficient food packaging materials [41]. Similarly, blending of neem oil and curcumin in poly lactic acid based composite films showed the improved antimicrobial and antioxidant activities, making them attractive choices for active food packaging applications [44]. Recently, areca fiber woven mats were used to formulate araldite epoxy resin adhesive based bionanocomposite blended with neem oil and nano silica, and the developed bio-nanocomposite had high thermo-mechanical strength, improved antimicrobial activities, and is a promising material to be used as food storage container [45]. Nanoemulsion of neem oil also has several advantages for applications. Approaches and various methods for preparation of nanoemulsion in sustainable food packaging and coating, and it is considered as a good alternative to conventional materials and practices. Neem oil nanoemulsion in pectin matrix showed improved mechanical properties, reduced stiffness, water vapor permeability, increased extensibility, and effective antifungal activities against Aspergillus flavus and Penicillium citrinum [42]. The neem oil and its nanoemulsion in biopolymer based composite food packaging films and coatings, as summarized. Migration of active agents may occur from packaging to food items through direct contact or through packaging headspace. Several factors such as storage temperature, duration and area of the package in contact with the food item, composition of the food, concentration of the active agents, and the biopolymeric material may affect the degree of migration of the bioactive agents from the package into the food item [46]. Encapsulation technology has been widely used by cosmetics and pharmaceutical industries to deliver the active agents or flavor compounds in the product in a controlled manner to improve their functionalities [47]. Encapsulation of neem oil improves bioactivity, bioavailability and stability of the active agents, and achieves targeted delivery along with controlled release, making it more effective functionality in food preservation and packaging [48]. Microencapsulation of neem oil in pitaya (Stenocereus pruinosus) fruit coating resulted in slow release of azadirachtin from the primary packaging, and improved the fruit shelf life for up to 15 days in refrigerated (10 ◦C, 80% RH) storage [40].

D) Cosmetics

Neem is perceived in the Indian key as a beauty aid. Neem has been used as a raw source of material in the beauty and wholesomeness of sedulity since time old. Given its wide range of medicinal parcels, neem is considered to work cautions for the skin and hair. Hence, it is used in the manufacture of a number of cosmetic products. Salanin and nimbin are the other major active implicit bioactive mixes that can be used for product development. Nimbidin which is natural extract attained by neem seeds has set up to be effective in the treatment of skin conditions analogous as eczema, furunculosis, arsenical dermatitis, scabies and seborrheic dermatitis. Implicit cosmeceutical operations include antibacterial, antifungal, antiparasitic, nonentity repellant, anti pediculosis phrasings for topical use in skin and hair care. Neemoids is a free flowing pale brown to pusillanimous brown cream attained from cold pressed Neem seed painting oil used in phrasings including creams, plasters, hand/ body washes, detergents, oils and related products. The bark is a rich source of tannins, gallic acid, catechin, epicatechin and polysaccharides. All of these ingredients are known to have antibacterial, antioxidant, anti-inflammatory, immunomodulatory and possibly, antitumor effects [50]. Leaves contain nimocinol and sulphuric compounds with insecticidal and antifungal effects. The peel contains meliatetraolenone, sesquiterpene, azadirone and benzopyranoids which have insecticidal and antibacterial properties[50]. Plants have substances called secondary metabolites that can develop antibacterial, antifungal and/or antioxidant activities (such as glycosides, alkaloids, flavonoids, saponins, among others), and can be also part of a defense mechanism against pathogens [49]. Such phytochemicals are extracted according to the molecule polarity and the characteristics of the vegetable part used. Thus, studies with methanolic extracts from A. indica leaves inhibited the action of Bacillus, while oils from seeds, bark and leaves could inhibit the growth and/or viability of Gram-negative and Gram-positive bacteria [49]. Among Gram-positive bacteria, we highlight the strains of M. pyogenes, Streptococcus mutans and Staphylococcus aureus, which are commonly found on the skin's surface.

Moreover, gedunin has antifungal activity and deoxygedunin has moderate antibacterial action, both of which were isolated from Neem seed oil [49]. *A. indica* leaf and seed grain extracts were effective against the human fungi *Candida, Geotrichum, Epidermophyton, Trichophyton, Microsporum* and *Trichosporon* [49].

Following are the daily usage of neem extracts

1) Face Wash- At present, a number of brands sell face marshes invested with neem extracts. This is due to the reason that neem is believed to relieve blights and acne and help promote clear skin [52].

2) Face Scrub: Neem is also used in face diminutives to remove impurities and oils. It can remove bacteria and pustules from the face and help the circumstance of acne [52].

3) Anti-Dandruff Shampoo: Multitudinous brands are dealing detergents invested with neem extracts, at the present day. Neem extracts present in detergents help in fixing dandruff, sanctification of dead cells, and preventing short crowns [52].

4) Face masks invested with neem are common at moment's request. Neem present in face masks help in keeping the skin impeccable and radiant [52].

5) Neem face wipes are affordable and amazingly saturated. Neem invested face wipes help in removing impurities and revitalizing the skin. They help to restore the natural radiance of the skin [52].

6) Skin Toner- Neem invested skin colors to help in guarding the skin from sun tanning and removing bruises. These colors clean the pores of the skin and remove face impurities, including makeup [52].

7) Hair oil painting oil Neem- predicated hair oils are generally set up at the request moment. These oils help relieve dry crowns and dandruff. They also stimulate hair follicles, leading to enhanced hair growth [52].

8) Cleanser/ Body Wash- A number of organic as well as marketable cleansers set up at the moment, are packed with neem extracts. These cleansers and body washes generally have a herbal aroma and help keep skin moisturized and bacteria-free.

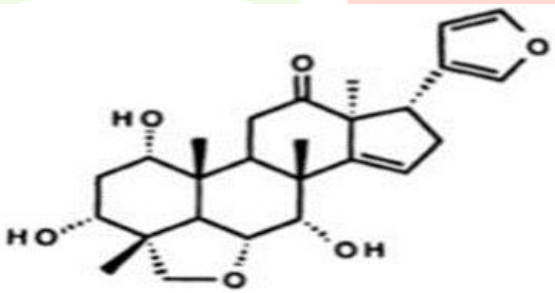

Fig.2 : Structure of Nimbidin[20]

The undiluted neem seed oil was tested against various strains of bacteria, to yield zones of inhibition as shown in Figure 3.

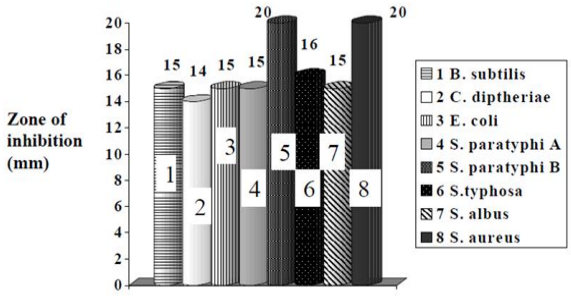

Fig.3 : Inhibitory effect of Neem oil against bacterial cultures

V) Chemical and functional properties of neem

Neem oil is an important traditional medicine and it has been used in agriculture for decades. Neem oil is a natural vegetable oil obtained mainly from seeds, kernels, and fruits of neem tree. Sometimes, it is also obtained in less quantity from other parts of the tree such as flowers and leaves but the yield is maximum from seeds, particularly from its kernel and not from its leaves after 3-months of storage. The yields of neem oil extracted from seed and kernel are about 18–25% and 42–50%, respectively[13] . Many methods have been used for extraction of neem oil, the most common among which are mechanical pressing, solvent extraction, and supercritical fluid extraction. In the mechanical extraction method, neem oil is extracted by mechanical crushing of the seed at controlled or cold temperature. Mechanical extraction technique is most commonly used because it is convenient, solvent-free, economical, and about 82% of neem oil is extracted using this method. Although, the oil extracted by this method is of poor quality and low market value due to low azadirachtin content, presence of significant amounts of water and metal that makes the oil turbid and impure.

Neem oil consists of more than 300 biologically active compounds, of which the major constituents are triterpenes known as limonoids (sal- annin, nimbin, nimbinin, meliantriol, azadirachtin, quercetin, etc.) , antioxidants, fatty acids, and triglycerides, etc.. The color of neem oil varies from yellow brownish , dark brown, golden yellow, reddish brown, greenish brown to bright red. Neem kernel oil is also a major source of fatty acid, and mainly composed of oleic acid (44.98%), stearic acid (21.26%), palmitic acid (16.78%) and linoleic acid (14.18%) [13] . Neem oil contains limonoids, calcium, etc [13].

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