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ANTIFUNGAL ACTIVITY OF NEEM SEED OIL FRACTIONS AGAINST CANDIDA ALBICANS

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Abstract

Neem (Azadirachta indica) seed oil has a long record in traditional practice, and fractions prepared from it are often proposed as antifungal agents against Candida albicans. This narrative review brings together published work on the chemistry of neem seed oil, the way its fractions are prepared, and the in vitro evidence of activity against Candida and other fungi. It also covers susceptibility and biofilm methods, toxicity reports and formulation questions. The literature points to limonoid-rich and fatty acid fractions with modest, reproducible inhibition, but testing is poorly standardised, solvent controls are inconsistent, and in vivo data are scarce. The review reports no new data and sets out priorities for better-designed studies.

1. INTRODUCTION

Candida albicans lives quietly on the mucosa of most healthy people, in the mouth, gut and vagina, and for most of them it never causes trouble. When host defences slip, through neutropenia, broad-spectrum antibiotics, diabetes, indwelling catheters or dentures, the same organism turns invasive. Candida species remain among the commonest causes of bloodstream infection in hospitals, and mucosal disease is far more frequent still [1, 2]. Vulvovaginal candidosis alone affects most women at least once, and a sizeable minority go on to suffer recurrent episodes [3]. Device-related infection adds another layer, because yeast cells settle on silicone, acrylic and catheter surfaces and form biofilms that are hard to clear [4].

Treatment rests on only a few drug families: polyenes, azoles, echinocandins and flucytosine [5]. Each has limits. Azoles are

fungistatic against Candida, and resistance builds through target-gene mutation, overexpression of *ERG11* and upregulated efflux pumps [6, 7]. Echinocandin resistance is less common in *C. albicans* but is documented, and the global picture is worrying [8]. Against that background, plant-derived products have drawn steady interest, partly because they are cheap and familiar and partly because they may act at sites different from the usual drug targets [9]. This review asks what the published literature says about one such candidate, the oil pressed from neem seed and the fractions prepared from it, and how far it can be trusted as an anti-Candida material. It reports no new experimental data. Readers who want to judge the claims for themselves will find the key questions in the sections that follow: what exactly was tested, how, against which strains, and with what controls.

Neem leaf, bark and seed preparations have been studied for decades, and the weight of that literature is easy to underestimate. Reviews describe anti-inflammatory, antipyretic, hypoglycaemic, antiulcer and antimicrobial effects, and attribute many of them to the limonoids and polyphenols of the plant [10, 11]. The difficulty is that a large share of this work involves crude extracts of unstated composition, which makes it hard to say which constituent does what. The antifungal findings follow the same pattern. They are numerous and mostly positive, but they sit on uneven foundations, and the move from a zone of clearing on an agar plate to a usable anti-Candida product is longer than the promotional literature suggests [12, 13].

2. NEEM SEED OIL AND ITS FRACTIONS

Cold-pressed neem seed oil is a yellow to brown liquid with a sharp garlic-like smell. Most of its weight is triglyceride, built mainly from oleic, palmitic, stearic and linoleic acids, with a smaller unsaponifiable portion that holds the bioactive terpenoids [12, 14]. Those terpenoids are limonoids, highly oxygenated modified triterpenes, and the best known are azadirachtin, nimbin, salannin, nimbidin and gedunin. Azadirachtin earned its fame as an insect antifeedant rather than as an antifungal, and it is present in relatively small amounts in the oil of any given lot [14]. Composition varies with the tree, the season, the age of the seed and the way the oil is extracted, which is a recurring difficulty when results from different laboratories are compared [13, 15].

Fractionation is usually carried out in a handful of familiar ways. Hexane-extracted or pressed oil may be partitioned between a non-polar solvent and aqueous methanol so that triglycerides stay behind and the limonoid-rich portion moves into the polar layer. Alternatively the oil is saponified, the soap removed, and the unsaponifiable matter run through silica gel columns with gradients of hexane and ethyl acetate. Antifungal screening of the resulting fractions guided isolation of active constituents from neem seed oil in work reported by Govindachari and colleagues, who followed activity through successive columns rather than testing crude oil alone [16]. That bioassay-guided approach is still the most defensible one, because it ties a biological effect to a chemical fraction.

Structure helps to explain why limonoids are expected to interact with fungal cells. They are bulky, oxygen-rich triterpenoids with a lipophilic core, so they partition readily into lipid bilayers, and the fungal membrane with its high ergosterol content is a recognised vulnerable site for several antifungal classes [17]. Polyenes bind ergosterol directly, and azoles deplete it. A limonoid need not do either to be harmful to the cell, since insertion into the bilayer can change packing, permeability and the function of embedded enzymes. This is inference from physical chemistry, yet it is consistent with how other plant terpenoids behave, and it offers testable predictions about leakage of intracellular contents [12, 14].

Not every effect seen with whole oil belongs to the terpenoids. Free fatty acids, which are released when oil ages or is hydrolysed, have their own antimicrobial activity. Short and medium chain acids and some unsaturated long chain acids were shown to inhibit oral microorganisms, including *C. albicans*, in a study of lipid antimicrobials [18]. Since neem oil is rich in oleic and linoleic acids, a plain fatty acid fraction may account for part of any observed inhibition. This is rarely controlled

for. A study that compares a limonoid fraction with a matched fatty acid methyl ester fraction would settle the question far more cleanly than yet another crude extract.

Quality control for a plant-derived material is a field of its own. Compendia of official methods for oils and fats, such as the AOCS collection, set out routine tests for identity and quality, for instance acid and peroxide values that indicate rancidity, alongside checks for moisture and impurities [19]. Adulteration is a recognised problem in the neem oil trade, where seed oil may be blended with cheaper vegetable oils. A fraction cannot be meaningfully compared across studies until its identity and basic quality have been established by such methods, and few antifungal papers report them [13, 19].

Standardisation is the unglamorous problem underneath all this. Azadirachtin content is routinely measured by HPLC, but it is a poor marker of antifungal quality, and few papers report chromatographic fingerprints for the fractions they test [13, 15]. Without a fingerprint, a result reported from one batch says little about the next. Storage matters too, because limonoids degrade under light and heat, and peroxidation of the oil changes its fatty acid profile within months in warm climates. Reporting the oil source, extraction method, storage conditions and a basic chemical profile would let readers judge which fractions are in fact comparable.

3. ANTIFUNGAL ACTIVITY AGAINST CANDIDA

The antifungal literature on neem is wider than the Candida literature, and it helps to read the two together. Seed oil and leaf preparations have been reported to inhibit the growth, sporulation and toxin production of filamentous fungi in culture [20], and neem was found to slow the growth of dermatophytes in a study of clinical isolates [21]. Leaf extracts and the limonoid nimonol were active against several human pathogens, Candida among them, in a comparison with standard drugs [22]. Findings across these papers are summarised in table 1. The pattern is consistent in direction, which is encouraging, but the potency is usually modest when set beside an azole or polyene tested in the same system.

Table 1: Published findings on neem preparations and antifungal or anti-biofilm activity

Preparation or topic	Reported finding	Ref.
Neem seed oil bioassay-guided fractionation	Antifungal constituents isolated from seed oil by following activity through successive chromatographic steps	[16]
Neem oil and leaf extract Penicillium	Growth, sporulation and toxin production of the mould reduced in culture	[20]
Neem extract dermatophytes	Growth pattern of clinical dermatophyte isolates altered in culture	[21]
Aqueous extract C. albicans on resin	Lower hydrophobicity, adhesion and biofilm formation on composite resin	[23]
Leaf extracts and nimonol	Activity against several human fungal pathogens, compared with standard drugs	[22]
Fatty acids oral microorganisms	Medium and long chain acids inhibit oral organisms including C. albicans	[18]
Broth microdilution	Reference methods for yeasts give reproducible MIC values for soluble drugs	[24, 25]
Plate-based biofilm model	Reproducible 96-well method for biofilm formation and susceptibility testing	[26]
Plant extract MIC criteria	Quality standards suggested for activity claims, with controls and selectivity	[27, 28]
Margosa oil ingestion	Encephalopathy and Reye-like illness in children after oil poisoning	[29, 30]

Evidence specific to *C. albicans* is thinner. An aqueous neem extract reduced hydrophobicity, adhesion and biofilm formation by *C. albicans* on composite resin, which is a different claim from killing planktonic cells and arguably a more useful one for denture and device surfaces [23]. Antifungal compounds identified from the seed oil were tested against yeast and mould, and the bioassay-guided work supplies the nearest thing to a defined active fraction [16].

Most other reports use crude oil or crude extract, tested by disc diffusion, so the zone of inhibition is the only readout. Zone size depends heavily on how well a lipophilic material diffuses through agar, and it should not be read as potency.

How might these fractions work? Several mechanisms have been proposed and none is firmly established for *Candida*. Lipophilic limonoids and fatty acids may intercalate into the plasma membrane and alter its fluidity or permeability, which is plausible for an organism whose membrane depends on ergosterol [17]. Interference with the yeast-to-hypha switch is another suggestion, important because filamentation is tied to tissue invasion and to the peptide toxin candidalysin [31, 32]. Reduced adhesion fits the biofilm finding above [23]. These ideas are reasonable, yet they come mostly from inference. Few studies have examined membrane integrity, ergosterol content, reactive oxygen species or gene expression after treatment, and without that work the mechanism stays a hypothesis.

Not every useful agent needs to kill the yeast. *C. albicans* causes damage largely through hyphae, which invade epithelium and secrete candidalysin, a peptide toxin that injures host cells and drives inflammation [31, 32]. Adhesion to epithelial and prosthetic surfaces comes first, and biofilm formation follows [33]. A material that reduces adhesion or filamentation without killing could lower virulence while exerting less selective pressure for resistance, an argument sometimes made for anti-virulence strategies. The reported effect of neem extract on adhesion and biofilm fits that idea [23], though no study has yet tied neem seed oil fractions to candidalysin release or to epithelial damage in an infection model [32].

It is fair to ask what 'modest' means here. Fluconazole and amphotericin B inhibit susceptible *C. albicans* at concentrations of well under a few micrograms per millilitre, whereas crude plant preparations are generally tested in the range of hundreds to thousands of micrograms per millilitre [5, 28]. A fraction that needs such concentrations is unlikely to be given systemically. Topical use is a different matter, since a mouthrinse, gel or denture soak can deliver high local concentrations, and the relevant benchmark is then the pharmacology of other topical agents and not that of systemic drugs [9, 34]. Framing neem work this way keeps expectations in proportion.

A useful way to see the shape of the evidence is to count where it sits (fig. 1). Entries concentrate on leaf and seed preparations tested for growth inhibition, with only a few on biofilm and adhesion, and still fewer on safety. That imbalance matters. A candidate intended for oral or vaginal use needs information on anti-biofilm action, toxicity to mucosal cells and stability in formulation, and these are precisely the categories with the fewest entries. Reviews of antifungal natural products make the same point more generally: much screening, little development [9, 28].

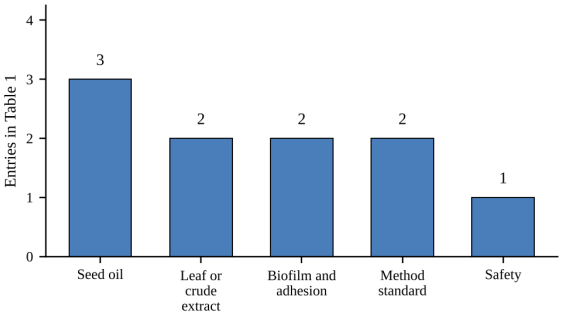


Fig. 1: Number of entries in table 1 by evidence category

Combination effects deserve a mention. Several natural products are reported to lower the inhibitory concentration of fluconazole against resistant Candida when added at sub-inhibitory doses, probably by perturbing the membrane or efflux. If neem fractions behaved similarly they could have value as adjuvants rather than stand-alone agents, but published checkerboard data for neem seed oil fractions are, to the knowledge of this review, scarce. The idea remains attractive in principle, given that resistance mechanisms in Candida centre on efflux and target alteration [6, 7].

4. EXPERIMENTAL METHODS AND THEIR PITFALLS

Reproducible susceptibility testing starts with a reference method. The CLSI broth microdilution protocol for yeasts and the EUCAST definitive document both specify medium, inoculum density, incubation time

and endpoint reading for antifungal drugs [24, 25]. They were written for soluble synthetic drugs, and applying them to oils needs adjustments that should be stated openly. Many neem papers skip them, report only agar diffusion, and compare their result with a drug disc of unrelated potency. An MIC determined by broth microdilution, ideally with a minimum fungicidal concentration (MFC) alongside, gives a number that another laboratory can reproduce and a clinician can interpret. Reporting the inoculum size, medium and reading time in every paper would remove much of the guesswork when results are compared [24, 25].

Solvent choice is the next trap. Neem oil does not dissolve in water, so investigators use dimethyl sulfoxide (DMSO), ethanol or a surfactant such as polysorbate 80. These vehicles can inhibit Candida by themselves at higher concentrations, and surfactants alter membrane behaviour in just the way a lipophilic fraction is supposed to. Every experiment needs a vehicle control at the highest concentration used, and the final solvent percentage should be reported. Turbidity is a second nuisance, because emulsions cloud the wells and confuse optical density readings. Colorimetric indicators such as tetrazolium salts overcome part of that problem, and the microplate approach with a resazurin indicator, described by Sarker and colleagues, has been widely adopted for plant extracts for exactly this reason [27, 35].

Biofilm assays need as much care. The 96-well plate model, in which biofilms are grown on the plastic surface, treated and then measured by metabolic dye reduction, is simple and has been shown to be reproducible across laboratories [26]. Candida biofilms differ from planktonic growth in their architecture, matrix and tolerance to drugs, and a fraction that inhibits the free-floating cell may do little to a mature biofilm [33, 36]. Papers should therefore separate prevention of attachment, inhibition of biofilm development and disruption of a pre-formed biofilm, because these have different clinical meanings. They are often merged under the single heading of anti-biofilm activity.

Choice of test organism deserves more thought than it usually gets. Reference strains give comparability, but a panel that includes fluconazole-resistant clinical isolates, non-albicans species and strains with known efflux overexpression would say much more about the potential of a fraction [7, 8]. Incubation conditions also shift outcomes: hyphal growth is promoted at 37 °C in serum or neutral pH, while yeast-form growth dominates in acidic medium at lower temperature [31]. An agent that blocks filamentation may therefore look active in one medium and inert in another. Reading endpoints at 24 and 48 hours, as the reference methods allow, helps to separate delayed growth from true inhibition [24, 25].

5. SAFETY AND FORMULATION

Neem has a reputation as a gentle herbal product, and that reputation needs qualifying where the oil is concerned. Accidental or deliberate ingestion of margosa oil has caused vomiting, metabolic acidosis, seizures and encephalopathy in children, with a Reye-like picture described as early as 1981 [30]. Toxicological reviews of neem-derived products conclude that low-level exposure through formulated products appears tolerable, yet they flag the absence of good long-term data and the variability of the material [29]. For topical or oral rinse use, a fraction with reduced oil content and a defined limonoid profile is safer ground than whole oil, though that proposition is still to be tested.

Formulation could decide whether any of this work reaches patients. Oil fractions suit emulsions, nanoemulsions, gels and mucoadhesive systems better than simple solutions, and denture-associated stomatitis is a natural target, since it is common, driven by Candida biofilm on acrylic and managed poorly by repeated antifungal courses [34]. A neem-containing denture cleanser or liner coating would exploit the reported effect on adhesion to resin [23]. Vaginal gels raise a different problem, namely compatibility with lactobacilli and with vaginal pH, which no neem study seems to have addressed in depth [3]. Stability and sensory acceptability, in particular odour, also need consideration.

Clinical evidence is the weakest link of all. To the knowledge of this review, there are no

well-controlled trials of neem seed oil fractions for candidosis, and the human data available concern other neem preparations or other conditions [11, 15]. Standard care for oropharyngeal and vulvovaginal disease is well established, with topical azoles or polyenes, and any new product would need to show benefit against that comparator, or in patients who fail it [3, 5]. Recurrent vulvovaginal candidosis and denture stomatitis, both frustrating to manage, are the more realistic settings in which an adjunct could prove its worth [3, 34].

Regulatory expectations for any topical antifungal include irritation and sensitisation testing, assessment of impurities and demonstration of batch consistency. Natural products receive no exemption in principle, and a standardised fraction would face the same scrutiny as a synthetic compound. Neem-derived pesticides have been assessed under that kind of framework, and the review of their safety found acceptable margins for several uses but also noted gaps in reproductive and chronic toxicity data [29]. For a medicine, those gaps would have to be closed, and quality controls of the type set out in official oil methods would apply [19].

6. LIMITATIONS AND FUTURE DIRECTIONS

The weaknesses of the present evidence are fairly plain. Most studies are single-laboratory, in vitro, and use poorly characterised oil or crude extract. Strains are seldom identified beyond the species level, and clinical isolates with known azole resistance are rare in the test panels. Positive controls and vehicle controls are unevenly applied, and potency is typically described in terms of inhibition zones. Published reports are also vulnerable to the usual bias towards positive findings, since a neem fraction that fails to inhibit Candida seldom gets written up. Readers should therefore treat reported activity as a lead, not as proof [9, 28].

Several steps would move the field forward. First, fractions should be prepared by reproducible procedures and fingerprinted chromatographically, with matched controls for fatty acids and solvents. Second, susceptibility should be determined by broth microdilution against reference strains and resistant clinical isolates, with fungicidal endpoints [24, 25]. Third, biofilm work should distinguish prevention from disruption [26, 33]. Fourth, cytotoxicity against oral and vaginal epithelial cells should be measured alongside activity so a selectivity index can be calculated [27, 29]. Only then would animal models of oral or vaginal candidosis be justified. Publication of negative results from such studies would be just as informative as the positive ones, and would help the field avoid repeating dead ends [9, 28].

A broader point concerns how natural product leads progress. Many plant extracts show activity in the first screen and then stall, because the active principle is a minor constituent, is unstable, or is not selective enough for the host cell [9, 27]. Neem is no exception. Its attraction lies in familiarity and availability, which help with acceptance, but the same features can hide a lack of rigour. Investigators who commit to isolating and characterising the active limonoid or fatty acid, following the approach of earlier bioassay-guided work, give the field its best chance of producing something that can be developed [16].

7. CONCLUSION

Published work suggests that neem seed oil and the fractions made from it can inhibit Candida and other fungi in the laboratory, and that part of this effect may come from limonoids and part from plain fatty acids [16, 18]. The most clinically relevant signal is the reduction of adhesion and biofilm on denture resin, although it was obtained with an aqueous extract and not with oil [23]. Potency is usually modest, mechanisms are inferred, and standardisation is poor [13, 27].

For now, neem seed oil fractions are best regarded as leads that merit careful follow-up, not as ready therapeutic agents. Better-defined fractions, reference susceptibility methods, honest vehicle controls and early toxicity testing would make the next round of studies more useful [24, 29]. If these are in place, formulations aimed at denture stomatitis or topical candidosis are a realistic goal for the coming

years [34].

8. ABBREVIATIONS

CLSI-Clinical and Laboratory Standards Institute, DMSO-Dimethyl sulfoxide, EUCAST-European Committee on Antimicrobial Susceptibility Testing, HPLC-High-performance liquid chromatography, MIC-Minimum inhibitory concentration, ERG11-Ergosterol biosynthesis gene encoding lanosterol demethylase, MFC-Minimum fungicidal concentration, WHO-World Health Organization

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10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

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