

The Unexplored Anticaries Potential of Shiitake Mushroom

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ABSTRACT

Keeping an eye the escalating costs of dental services, the treatment cost of the consequences of dental caries can be reduced to manageable proportions by preventive measures aimed at decreasing the prevalence. One such measure is by increasing the consumption of caries preventive foods. Recently, there has been an upsurge of interest in mushrooms not only as a healthy food but also as a caries preventive food. The most common type of mushroom, *Lentinula edodes* also called as shiitake, is studied in-depth for its oral health benefits. The cultivation of shiitake dates way back to 1100 A.D. during the rule of Sung dynasty which is replaced by more modern and efficient sawdust substrate log cultures lately. Shiitake mushroom extract can be isolated in various forms such as freeze dried, oil, and ethyl acetate extracts. Various biologically active compounds such as erythritol, copalic acid, adenosine, carvacrol, and many more are responsible for this mushroom's antimicrobial activity. Anticariogenicity can be attributed to the induction of the detachment of cariogenic microorganisms from hydroxyapatite, changes in cell surface hydrophobicity, bactericidal activity, and disruption of signal transduction in *Streptococcus mutans* as proved through various *in vivo* and *in vitro* studies. Apart from these benefits, it has tremendous potential to be used as an antioxidant, anticancer, antigingivitis, antifungal, and antiviral agent. The one and only known adverse reaction due to shiitake mushroom consumption is the eruption of pruritic erythematous papules termed as shiitake dermatitis. This review highlights the unexplored anticaries potential of one such useful bioactive metabolite-shiitake mushroom.

Key words: Extract, functional foods, oral health, shiitake mushrooms

INTRODUCTION

Dental caries has been defined as "A localized, posteruptive, pathological process of external origin involving softening of the hard tissue and proceeding to the formation of a cavity."^[1] Dental caries affects 60–90% of schoolchildren and the vast majority of adults and is the most prevalent oral disease worldwide. A WHO estimation of global decayed, missing filled teeth for just the 12-year-old children reported that 200,335,280 teeth were either decayed, filled, or missing among the 188 countries included in their global database.^[2,3] The treatment of the consequences of dental caries is time consuming and expensive, and the costs of medical and dental services are constantly rising making it obvious that dental caries cannot be managed by the treatment alone. The problem can be reduced to manageable proportions only by preventive measures aimed at decreasing the prevalence of the disease.^[1]

Traditionally fluorides were considered to be the cornerstone in caries prevention. However, fluorides used for caries prevention have its limitations.^[4] In the light of these limitations, nonfluoride caries preventive agents gained importance. These newer nonfluoride topical remineralizing agents contain calcium and/or phosphate that aim to increase the bioavailability of these minerals at the tooth surface in order to enhance remineralization.^[4]

Recently, development of dental caries has been inhibited by the consumption of specific foods. Today, a number of foods and food components are known to act potentially as anticaries agents such as green tea, apple, red grape seeds, nutmeg, coffee, mushroom, cranberry, garlic extract, cocoa extracts, and propolis.^[5]

In the recent times, there has been an upsurge of interest in mushrooms not only as a healthy food which is rich in protein but also as a source of biologically active compounds of medicinal value. One such property of importance is its anticariogenicity. The most common type of mushroom is *Lentinula edodes* also called as shiitake. It is one of the most popular edible mushrooms in the world, production globally being second only to the button mushroom.^[6]

Very few studies have been conducted on caries inhibitory effect of this shiitake mushroom extract. Hence, there is a scope for further research as shiitake has not been assessed in-depth for its oral health benefits. Thus, this review in its attempt to disclose the unexplored potential of shiitake mushroom discusses the cultivation history, preparation of the extract, biologically active compounds in the extract, studies of anticariogenicity, other oral health benefits, adverse reactions, and future directions.

Cultivation History

It has a fascinating history when it comes to its cultivation, being first discovered in China by a legendary figure known as Wu Sang Kang. He began his discovery while hunting and collecting mushrooms for food in the wild forests of the mountainous Lung Skyr village in

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the Southwest province bordering Fujian and also figured out how to grow wild shiitake during the rule of Sung dynasty in 1100 A.D.^[7] He noticed that shiitake were found on fallen logs in the woods and on cutting the logs, the mushrooms grew in a larger and better way; springing up profusely like flowers. This is said to be the origin of the “shocking method” in shiitake log cultivation.^[7] The Chinese growers then introduced the application of pure culture spawn in growing shiitake logs. The third historical milestone was the invention of sawdust substrate cultivation by a cylindrical method which was improved by Z.W. Peng in China in 1983.^[7] The latest development in the USA is the use of much larger sawdust substrate blocks in sealed polypropylene bags with microporous filters as breathing windows. This type of cultivation may increase the yield three to four times to that of natural log cultivation.^[8,9]

Preparations of Shiitake Mushroom Extract

Various forms of this mushroom extract have been prepared to be used in various studies to evaluate its beneficial effects on oral health. These include a freeze-dried extract, oil extract, aqueous extract, and ethyl acetate extract.

The low molecular mass (LMM) fractions (<5000 Daltons) of shiitake mushroom can be prepared by ultrafiltration of the crude homogenate using the Vivaflow 200 system (Vivascience AG, Hannover, Germany) equipped with a membrane 5000 MWCO polyethersulfone for ultrafiltration.^[10] About 70% and 50% (w/w) of the components present in the crude mushroom homogenate were detected in the ultrafiltrates and were sterilized using a 0.20 µm pore size membrane (Vivascience), then freeze dried and stored up to 3 months at -80°C. This produces a freeze dried shiitake extract.

The oil extract of shiitake mushroom, a popular food product with certain medicinal properties, was isolated from finely ground dried shiitake mushrooms by hydrodistillation for 3 h and subsequently dried over 99.0% anhydrous sodium sulfate.^[11]

Dried shiitake (200 g) was immersed in 300 ml water at 4°C overnight. Wet shiitake was ground in a mixer and mixed with chloroform (1600 ml) with stirring at 4°C overnight. The chloroform and insoluble materials were then separated by filtration or centrifugation. The chloroform extract was evaporated under reduced pressure to yield light yellow oil (876 mg). The insoluble materials were then mixed with water (1000 ml) with stirring at 4°C overnight. The suspension obtained was filtered or centrifuged to remove the insoluble matter; the aqueous supernatant was concentrated under the reduced pressure to 200 ml. The aqueous extract was lyophilized and re-suspended with water completely. This is known as the “aqueous extract.”^[12]

A similar process is followed to derive the ethyl acetate extract till the supernatant formation after which concentrated preparation was then extracted by vigorously shaking with 200 ml ethyl acetate for three times. The organic layer was separated and evaporated under reduced pressure to yield a light yellow material (114 mg). This is the “ethyl acetate extract.”^[12]

Biologically Active Compounds in the Extract

A variety of biologically active compounds is present in the extract obtained from this mushroom which has potent antimicrobial activity. These include the following:

- Erythritol^[13]-1,2 and 3,4-butanetetrol; which has 70–80% sweetness to that of sucrose
- Sesquiterpenes, steroids, anthraquinone, benzoic acid derivatives, and quinolones;^[13] present in LMM fraction of shiitake extract

- Copalic acid;^[14] a metabolite of sesquiterpenes
- Adenosine;^[15] present in LMM and high molecular mass fractions of shiitake extract
- Carvacrol;^[15] an aromatic monoterpene present in the oil extract of shiitake mushroom.

Studies of Anticariogenicity of Shiitake

A number of biological activities relevant to caries prevention have been identified through various *in vitro* and *in vivo* studies.^[11,13,16-19] These include induction of the detachment of cariogenic microorganisms from hydroxyapatite, changes in cell surface hydrophobicity, bactericidal activity against cariogenic microorganisms, and the disruption of signal transduction in *Streptococcus mutans*.^[19]

One of the major components of oral biofilm formed by cariogenic bacteria *S. mutans* and *Streptococcus sobrinus* is glucan. These microorganisms form biofilms on the tooth surface by attachment to the acquired pellicle on the enamel and adhesion to the tooth by cell to cell interactions.^[20] The shiitake extracts could reduce preformed biofilms in the presence of dextranase which is a water-soluble α-(1,6)-linked glucan. This dextran degrading enzyme, dextranase, has been used in the treatment to reduce the formation of dental plaque.^[20] An *in vitro* dental caries biofilm model was developed in combination with cariogenic microorganisms with dental hard tissue substratum (dentin or enamel) allowing modeling of frequent acid challenges by sucrose pulsing with a constant depth film fermentor. The amount of mineral loss quantifies anticariogenicity. This mineral loss is inhibited by low molecular weight (LMW) fractions of shiitake extracts *in vitro*.^[16]

This mushroom contains erythritol which is 1,2 and 3,4-butanetetrol which has 70–80% sweetness to that of sucrose. In the presence of erythritol, *S. mutans* and *S. sobrinus* showed no adherence to glass which suggests that this sugar is not utilized by the bacteria for the synthesis of glucans. Since it is not used by these bacteria nor is it metabolized to produce the lactic acid byproduct, it does not cause enamel demineralization.^[13]

Both high molar mass and LMM fractions inhibit *S. mutans* adherence to hydroxyapatite crystals, promoting the detachment of bacteria and inducing biofilm destruction.^[5] This is mainly carried out by a compound called adenosine. An *in vitro* study was conducted to characterize the bacterial receptors for the purified mushroom subfraction-5 in order to better understand the action of this subfraction on interbacterial and host interactions, *S. mutans* and *Prevotella intermedia* were the two bacterial strains used to study the same. Binding subfraction-5 to surface molecules of the above-mentioned bacteria may result in inactivation of their physiological functions. As a whole, the results indicated that at the molecular level, the bacterial surface alterations affected adhesion and biofilm formation.^[17] This extract has demonstrated an inhibitory effect on one of the virulence factors of *S. mutans* when specific pathogen-free rats were infected with *S. mutans* and fed with a cariogenic diet containing 0.25% shiitake extract and the controls were fed with a cariogenic diet without shiitake extract. A lower caries score was observed in the test group as the extract helped in reduction of firmly adherent plaque. This led to an increase in the incidence of non/loosely adherent plaque and a decrease in total plaque formation.^[18] A double-blind, three-leg, cross-over, randomized, controlled clinical trial was done to investigate the caries-preventive properties of LMW fraction of edible mushroom shiitake extract. Volunteers were asked to rinse twice daily with a solution containing LMW fraction of edible mushroom, placebo (negative control without active ingredients), or meridol (positive control) for 2 weeks with a 2 week washout period between each rinsing period. This trial assessed the shift in plaque pH

after a sucrose challenge, shift in the microbial flora, and the intraoral plaque accumulation. Results indicated that frequent rinses with shiitake reduced the metabolic activity of dental plaque.^[19]

The LMM fraction of shiitake extracts contains metabolites such as sesquiterpenes, steroids, anthraquinone, benzoic acid derivatives, and quinolones which inhibit the growth of *S. mutans*.^[13] This bacteriostatic action is carried out by the inhibition of DNA synthesis and elongation of bacteria with interrupted septa. These morphologic changes are similar to those observed in streptococcal thermosensitive temperature or exposed to inhibitory doses of β -lactam antibiotics.^[21] Copalic acid is a metabolite of sesquiterpenes which is an active compound displaying promising minimum inhibitory concentration (MIC) values (2.0–6.0 $\mu\text{g/mL}$) and has both bactericidal and bacteriostatic effects.^[14] In a separate *in vitro* study, the LMM fractions of shiitake mushrooms with a minimum of 2x concentrations inhibited the growth of *S. mutans* by inhibition of DNA synthesis. This bacteriostatic action was also confirmed by morphological effects by these fractions which showed the elongation of bacteria with interrupted septa.^[13]

An oil extract of shiitake mushroom has the best efficacy in terms of significant biofilm inhibition and disruption. This oil extract of shiitake mushroom at concentrations slightly above the MIC of *Porphyromonas gingivalis* showed a significant inhibition. Shiitake mushroom oil extract also contains carvacrol which has potent antimicrobial activity. Carvacrol is a known aromatic monoterpene shown to actively disintegrate the outer membrane of Gram-negative bacteria by releasing lipopolysaccharides from the bacterial cell wall and increasing membrane permeability.^[15] An *in vitro* study was carried out to investigate the biofilm inhibitory effect and disruptive efficacy of shiitake mushroom oil extract on four individual oral pathogens, namely *S. mutans*, *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans*, and *P. gingivalis* and on a mixed culture. This extract showed significant inhibitory and disruptive effects on *P. gingivalis*. Furthermore, *S. mutans* and multispecies biofilms also showed inhibition at its MIC.^[11]

The results of the *in vitro* and *in vivo* animal studies are difficult to transpose to humans because:^[5]

- The human oral environment could influence anti-bacterial activity
- It also has an influence on the virulence factor of the active compounds on oral pathogens and
- In most cases, food consumption probably allows insufficient contact time between the oral tissues and active compounds.

Other Oral Health Benefits of Shiitake

A significant oral health benefit of this mushroom is its antigingivitis effect. LMM fractions from shiitake mushroom interfere with the binding of *S. mutans* cells to hydroxyapatite and *P. intermedia* cells to gingival cells. LMM fractions bring about the detachment of odonto- and periodonto-pathogenic bacteria from preformed biofilms. The antimicrobial mode of action has been evaluated at MIC and an anti-biotic like mode of action has been described for the LMM fractions of mushroom. This activity strongly inhibited DNA synthesis and partially RNA synthesis with 50% reduction of protein synthesis.^[17] As a result of cell division inhibition, a certain degree of cell elongation has been observed in *S. mutans* while *P. intermedia* showed elongation in the form of filaments. The morphogenetic effects are similar to those obtained by treatment with antibiotics such as β -lactams or quinolones. A comparative study was carried out to determine the effectiveness of shiitake mushroom extract to that of the active component in leading gingivitis mouthwash, containing chlorhexidine, in an artificial mouth model. The total bacterial numbers, as well as numbers of eight key taxa in the oral community, were investigated over time and results indicated

that the shiitake extract lowered the numbers of some pathogenic taxa.^[22]

The disulfide derivative extracted with ethyl acetate has some antifungal activities. Based on its spectroscopic data, the chemical structure is shown to be bis-disulfide. Ethanolic mycelial extracts from shiitake also possess antifungal activity against *Paramecium caudatum*.^[23] Lenthionine was also found to be more sensitive to fungi as compared to bacteria.

The antioxidant effects of shiitake are due to L-ergothioneine (2-mercaptohistidine trimethylbetaine).^[24] This is due to the ability to trap reactive oxygen/nitrogen species and inflammatory mediators such as hydroxyl radicals, hypochlorous acid, and peroxy nitrite which is formed by the reaction of nitric oxide with superoxide.^[25] Reactive oxygen/nitrogen species and nitrous oxide are perceived as important signaling molecules generated during muscle contraction and involved in regeneration and adaptation of skeletal muscle to physical work. Thus, the extract of shiitake has the potential to modulate reactive oxygen/nitrogen species and nitrous oxide and enhances the skeletal muscle regeneration after intense exercise.^[25]

Shiitake mushrooms have also been reported to have cancer preventing properties due to the presence of “myochemicals” in them. These chemicals inhibit the growth of tumor cells which may result from induction of apoptosis. Lentinan from shiitake mushroom (0.5–1.0 mg/day, intravenously) has been used for adjuvant tumor therapy which leads to prolongation of survival time, restoration of immunological parameters, and improvement of life quality.^[26]

Antiviral activities were reported for mycelial culture medium of shiitake mushroom and sulfated lentinan completely prevented HIV-induced cytopathic effects. Antilipidemic effects were caused by a compound eritadenin which is a nucleotide derivative.^[25]

Adverse Reactions

The one and only known adverse reaction due to shiitake mushroom consumption is shiitake dermatitis. Consumption of raw or not well-cooked mushrooms may cause skin eruptions which usually occur 24–48 h after ingestion and are characterized by linearly arranged pruritic erythematous papules and plaques resembling flagellate pattern on the trunks and limbs.^[27] The compound which is suspected to cause shiitake dermatitis is lentinan which increases the secretion of interleukin-1 and causes vasodilatation.^[27,28] A complete reversal of these lesions takes place in about 2 weeks.^[29] The symptoms could be treated with antihistaminic drugs, topical corticosteroids and in severe cases, and oral corticosteroids for a short time.

CONCLUSION

Shiitake mushrooms have a great potential for the production of useful bioactive metabolites which belong to several chemical groups and have varied pharmacological effects. Dependent on increasing knowledge about chemistry, biotechnology, and molecular biology of mushrooms, a rapid increase in its application for medicinal purposes can be expected. There exists convincing evidence of this mushroom to be used as a functional food for the prevention of oral diseases and the betterment of oral health as a whole. Different extracts of shiitake have shown potent antibacterial and antifungal activities. Apart from these pharmacological benefits, it has tremendous potential to use as an antioxidant agent and also in prevention of cancer. From the oral health point of view, shiitake extracts have demonstrated caries preventive activities, reduction in oral biofilm formation, and antigingivitis effects.

The potential beneficial effects of shiitake mushroom have been studied on a small scale till date in various studies. However, further work needs to be done to isolate and identify the other active ingredients and explore suitable pharmaceutical delivery systems. The possibility of using the

isolated compounds in bioadhesive, time-specific and site-specific controlled release systems to enrich other foods could enhance its anticaries efficacy, overcoming the increasing problem of antibiotic resistance without altering oral microflora.

This review highlights the beneficial effects of shiitake mushroom in the disruption of oral biofilm, cancer prevention, and reduction of gingivitis. However, the most promising aspect being its anticariogenic effects. As such a new and exciting era of caries prevention through foods rich in biologically active components awaits us.

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Conflicts of interest

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