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Article

Evaluation of the Effectiveness of Sumac Extract on the Activity of A. Flavus Toxins in Nuts

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Abstract: The purpose of this study is to analyse the impact that the extract from the sumac plant (Rhus coriaria) has on the growth of Aspergillus flavus and how much it reduces aflatoxin content in nuts. The main byproduct of A. flavus is aflatoxins, which are toxic substances that can cause serious health issues, such as cancer, and negatively impact the economy. Current treatments for fungus are not very effective, so natural alternatives need to be explored. Sumac's multiple properties – antimicrobial/antifungal activity and antioxidant properties, combined with its high polyphenolic content (mainly tannin and flavonoid substances) – produce a potential solution. The efficacy of various concentrations (0.5%, 1%, & 2%) of sumac extract to inhibit the growth of A. flavus and decrease the amount of aflatoxin produced due to A. flavus in both food products were assessed through laboratory experiments. The results of the study indicate that the use of 2% sumac extract as an antifungal agent provided a greater than 88.3% reduction in the number of fungal colonies and more than 91.5% reduction in aflatoxin levels; both of these levels of inhibition are equivalent to those obtained from the synthetic compound sodium benzoate. Nutrition quality will also be preserved, shelf life will be extended, and aflatoxin levels will be decreased with the use of sumac extract as a food preservative. Additionally, researchers should focus on determining improved methods of applying sumac, as well as researching the shelf life of sumac and how effectively sumac can complement other naturally occurring antifungal compounds.
This research project advances the creation of sustainable food preservation approaches that use eco-friendly methods.

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1. Introduction

There are 250 species of Sumac in the genus Rhus which belongs to the family Anacardiaceae. The genus Rhus occurs in temperate and tropical regions of the globe. Species of the genus Sumac grow in areas that are not suitable for agriculture and provide traditional medicine to indigenous people. Therefore, the development of these plants as economic or commercial resources would not conflict with agricultural land use [1]. The native people of North America have used smooth sumac (R. glabra) to cure bacterial infections such as syphilis and gonorrhea, gangrene, and dysentery [2].

Researchers are focusing their efforts on the growth of the indigenous plant species R. coriaria or tanner's sumac. This particular species has been found to grow throughout the island groups of the Canary Islands and across the Mediterranean region all the way to the countries of Iran and Afghanistan where many people use it as a seasoning for food. After tanner's sumac has been processed into powder form by grinding up its dried fruit

along with some salt, that same powder has been used to make an herbal medicine that treats wounds and other ailments throughout both the Mediterranean and Middle East [3].

Sumac extract's biological properties have been studied in the literature of multiple research papers over many decades. Research about the sumac extract will use definitions from the literature; the lack of a comprehensive contemporary review about the biological activity for sumac extracts is apparent due to the need for the world to move forward with adopting sustainable bioproducts. The purpose of this review is to summarize the existing body of knowledge regarding the biological activities of sumac extracts, whilst proposing new possible directions for future research in terms of sumac extracts. Sumac extract analytical studies show that previous research has routinely focused on a small number of plants from the greater family of Rhus. From a research and development viewpoint, the timing of the publication of this review can greatly expand all of our possibilities for sourcing bioactive sumac extracts using green processing methods relying on promising research conducted regarding other plants from the Rhus genus [4]. Since the desirable products found in sumac can be sourced from areas in close proximity to their culture, there would be no need for long-distance travel of sumac products from sites of extraction to markets where sumac is sold. In addition, the genus Rhus has the potential to be a focus of green chemistry research due to its ability to be grown on non-arable land and produce biologically useful products and also because the genus has a wide distribution globally making it an appropriate candidate for research [4].

As a fungus that produces aflatoxins, Aspergillus flavus has caused many food foodborne illnesseswhen produces. Because aflatoxins have harmful effects on the immune system and can cause these diseases, any food contaminated with aflatoxins are a threat to both public health and the economy. A growing body of research is focused on finding natural antifungal preservatives that are safe for human consumption, primarily due to consumers being aware of the risks associated with food . Sumac, derived from Rhus coriaria, has received much of this research attention due to its high level of phenolic, flavonoid and tannin bioactives, which have been found to exhibit strong antifungal and other antimicrobial as well as antioxidant acti A saprophytic fungus, Aspergillus flavus produces the very toxic and carcinogenic secondary metabolites known as aflatoxins which contaminate many food products - such as nuts. Aflatoxin contamination poses a significant risk to food safety and human health, with toxic, immunosuppressive, and mutagenic properties [5], as well as to economic stability. With consumers becoming more aware of these risks; research has increased looking to discover natural antifungal preservatives. One type of natural antifungal preservative that has gained interest is sumac extract, Rhus coriaria, due to its bioactive compounds such as phenolics, flavonoids, and tannins with potent antifungal activity and also antimicrobial and antioxidant properties.vities.

A fungal species known as Aspergillus flavus produces the highly toxic secondary metabolites called aflatoxins which can contaminate many foods such as nuts. Aflatoxin contamination poses serious risks to food safety, human health, and the economy as it has several toxic, immunological, and mutagenic effects [5]. As awareness of these dangers has increased among the public, research has been conducted into safer, more natural antifungal preservatives. The use of sumac extract from Rhus coriaria is gaining attention due to its abundance of bioactive compounds (e.g., phenolics, flavonoids, and tannins), which exhibit strong antifungal, antimicrobial, and antioxidant effects.

Studies are now available to identify how certain plant-derived compounds fight against fungal infections while also preventing the fungi from producing harmful substances called mycotoxins (toxins produced by molds). Sumac extract has been shown to have some antifungal activity. For example, it affects the integrity of the fungal cell membrane, which limits the germination of the spores of the fungus and reduces the ability of fungi such as Aspergillus species (and other pathogenic fungi) to make mycotoxins. The

large amount of polyphenolic compounds in sumac can cause oxidative stress in fungal cells, resulting in the death of these cells (cellular suicide). Moreover, using sumac extract with standard antifungals can improve the efficacy of these antifungals and better equip them to work against fungal pathogens without contributing to any additional adverse side eff Sumac extract can be effectively used to make natural preservatives from nuts that help reduce aflatoxin contamination. Sumac extract is used to help preserve the shelf life of nut products and provide a safety measure against the possible aflatoxins that are present in nuts. More research needs to occur to find the proper concentration of sumac extract, how to apply it effectively, and to understand how food product types affect the antifungal efficacy of sumac extract. The goal of this study is to see if sumac extract can suppress the growth of A. flavus and inhibit the production of aflatoxins in nuts to prove it to be an effective alternative for the use of synthetic antifungals.ects.

2.1 The statement of the problem

Worldwide, Aspergillus flavus (A. flavus) contamination of nuts, including nuts that have been contaminated with aflatoxins (AFs), poses significant public health challenges and has significant economic implications. A large body of research has shown that (AFs) have carcinogenic, hepatotoxic and immunosuppressive effects, demonstrating the need for validated methodologies for determining food safety. The use of both fungicides and physical means of decontamination have serious limitations; fungicides are complex and often create toxicity or lose their efficacy over time. Plant derived naturally occurring antifungal substances provide new possibilities as eco-friendly alternatives to traditional methods. For example, sumac (Rhus coriaria) has demonstrated antimicrobial properties against fungi and contains antioxidant compounds that could inhibit fungal growth and the toxin production associated with the growth of A. flavus. Currently, there is very limited scientific information about the use of sumac extracts as a means to treat A. flavus contamination and prevent the production of toxins in foods such as nuts.

Scientists have initiated research to assess how sumac extract suppresses or inhibits A. flavus growth and development of toxins from this fungus to establish the potential for this agent as a naturally occurring food preservative.

Health risks associated with Aspergillus flavus contamination of nuts and their aflatoxins are significant and result in major issues. The purpose of these research results is to develop a novel food safety approach using sumac extract since this plant extract has been found to both inhibit fungal growth and prevent the formation of aflatoxins; therefore, it meets consumer expectations as a natural preservative while minimizing mycotoxin exposure.

3.1 The aim of the study

Researchers intend to develop a novel food safety technique to provide an alternate source of natural preservatives from sumac extract because of sumac's potential as a fungicide and blocker of aflatoxins and for meeting the consumer demand for natural preservatives while reducing the risk of mycotoxins. The combination of chemical fungicides and physical decontamination methods has significant disadvantages due to the potential toxicity of both chemical fungicides and the failure of the chemical fungicides to work effectively. Natural antifungal agents derived from plants offer promise as safe alternatives to conventional methods because they do not contribute to the introduction of new chemical agents and have environmentally sustainable characteristics. In addition to having fungicidal activity, a common medicinal plant, sumac (Rhus coriaria), has the additional property of having antioxidant activity, making it a good candidate for use as a fungal growth retardant and as an inhibitor of toxins. While there has not been enough published data in the scientific literature to prove the effectiveness of sumac extract against A. flavus and against the production of aflatoxins in nuts, researchers are investigating sumac extract's effect on A. flavus fungal growth and its effect on inhibiting the production of aflatoxins in nuts as a potential natural food preservative.

4.1 The importance of the study

This study is important because it explores the food safety issue related to Aspergillus flavus contamination, which leads to the production of aflatoxins. Aflatoxins can cause serious health problems and cause loss to the food industry as a whole. The purpose of this research is to evaluate the antifungal and anti-toxigenic properties of sumac (Rhus coriaria) extract so that eco-friendly, natural methods of preserving food can be developed and used to replace hazardoussynthetic antifungal preservatives. The research also created a new eco-friendly additive that enhances the safety and shelf life of nuts, forming a basis for the growing demand of the market for natural additives. Through this research, researchers, regulators, and the food industry can rely on the data available to them to use as evidence of control of mycotoxins using sumac extract and implement safer methods to protect food security and public health.

2. Methodology

The effects of sumac extract from Rhus coriaria on antifungal action and inhibition of toxin production against Aspergillus flavus, a fungus that contaminates nuts, have been tested in laboratories with controlled experiments done prior to the commencement of any in vitro study. After all of the aforementioned testing was completed, in vivo studies were performed. Using a randomized controlled method to conduct their evaluation of the sumac extract solution, researchers conducted comparisons between the sumac extract solution, (1) nuts with an A. flavus contaminant receiving antifungal treatment, (2) nuts with an A. flavus contaminant not receiving any antifungal treatment, and (3) control (no inoculation of an A. flavus contaminant) nuts. Each type of nut has been replicated three times in the study for both accuracy and statistics.

1.2 Sample Collection

1.2.1 Nut Sample Selection and Preparation

Local warehouses and markets provide nut samples (peanut, almond, and walnut) for various types of contamination. Before being sent to the laboratory via sterile methods to prevent outside sources of microorganisms, laboratory staff perform both physical and contamination testing of nut samples**.**

Laboratory staff have cleaned and sanitized all specimens before receiving them at the specified temperature-controlled facility until experimental inoculation occurs.

1.2.2 Fungal Strain Isolation and Identification

Researchers use both durian and Mexican chocolate to grow Aspergillus flavus from nature or from culture collections. Scientists are able to identify these isolates using the standard mycological technique of evaluating their morphology (colony pattern, spore characteristics) and/or the colour of both cultures and substrate media. Scientists confirm the identities of these isolates using a PCR method to amplify groups of genes related to A. flavus. Researchers then perform experiments to show the ability of the confirmed toxin-producer isolates to inhibit aflatoxin production.

1.2.3 Sumac Extract Preparation and Characterization

Acquisition of dried sumac fruit (from the species Rhus coriaria) has been approved by authorized botanicals producers, who use solvent (ethanol or methanol)-based maceration for extraction to produce the end solution of the ground plant material. Concentration of extract for final storage (to extend the life of bioactive components) occurs via rotary evaporation. The bioactive content in the extract is tested using spectrophotometric analysis for Total Phenolic Content (TPC) and Total Flavonoid Content (TFC). Evaluation of contained essential polyphenols and flavonoids (both antifungal in nature) is performed on HPLC (Agilent 1200 Series) at one of the research centres dedicated to pharmaceutical analysis in Iraq.

2.2 Experimental Procedure

2.2.1 Inoculation of Nut Samples with A. flavus

Nut samples inoculated with A. flavus spores were maintained in sterile conditions by utilizing phosphate buffered saline (PBS). The 25–30°C temperature range and humidity conditions facilitate proper fungal growth and subsequent production of aflatoxins.

2.2.2 Treatment Application and Incubation

Three concentrations of sumac extracts (0.5 percent, 1 percent, and 2 percent) will be used in the treatment of inoculated walnut samples. There will be a control group and a test group for each treatment. The control group will receive a standard antifungal agent (sodium benzoate) and the other groups will not receive any treatment at all. The samples in the treatment groups will receive 7 to 14 days of incubation time in order to assess their ability to support fungal growth and produce aflatoxins.

2.2.3 Assessment of Fungal Growth and Aflatoxin Inhibition

Fungal growth assessment relies on the counting of colony-forming units (CFUs) on selective plates combined with microscopic evaluations. The two techniques used for aflatoxin measurement include enzyme-linked immunosorbent assay (ELISA) combined with HPLC analysis. The research determines inhibition percentages through the examination of treated samples versus control groups .

2.2.4 Statistical Analysis

Data from these studies were collected in triplicate and analyzed using statistical software packages including SPSS and GraphPad Prism. A one-way ANOVA and post hoc tests (Tukey's test) were employed to determine differences between treatment groups at a significance level of $p < 0.05$, using means ± standard deviations (SD) for data presentation.

2.2.5 Ethical Considerations

This work implements ethical protocols of microbiological study by establishing biosecurity procedures to handle fungal strains along with chemical substances. The implementation of botanical extracts fulfills all current regulatory requirements as well as environmental protection standards.

3. Results

Table 1: Nut sample selection and preparation.

Nut Type	Source	Sterilization Method	Storage Conditions
Peanuts	Local Market	Ethanol Wash	4°C, Dry Storage
Almonds	Storage Facility	UV Sterilization	4°C, Dry Storage
Walnuts	Local Market	Autoclave	4°C, Dry Storage

Comment 1: By examining several nut types, the research will be able to determine what types of nut types create a variety of contamination scenarios. Through the use of a sterilizing technique, removal all microbial substances will allow for experimental Aspergillus flavus as the only contaminant. By controlling the temperature of the nuts before the experimental phase of the study, premature fungal growth will be avoided, and correct execution of this step will ensure accurate antifungal assessment results will be provided. Characteristics of different types of nuts can impact how different fungi colonize

the nuts, therefore will provide researchers knowledge relating to how differences in effectiveness of sumac extract treatments occur on individual nut varieties.

Table 2: Fungal Strain Isolation and Identification.

Source Material	Morphological Identification	PCR Confirmation
Durian	Colony Pattern, Pigmentation	ITS Gene Amplification
Mexican Chocolate	Spore Traits, Growth Rate	Aflatoxin Gene Detection

Comment 2: By combining morphological analysis and molecular testing techniques, there is a definitive way to identify strains of Aspergillus flavus. After initial morphological characterization based on pigmented colony development, PCR can be used for unsurmountable genetic markers in order to prevent a source of ambiguity within the results. This study utilizes both methods to verify that only pathogenic A. flavus strains remain in order to evaluate the efficacy of aflatoxin inhibition. By using varied materials, researchers are able to determine how fungal propagation & toxin production are impacted by the origins of the strain(s). This protocol further supports the integrity of the investigation by demonstrating that the resultant findings were produced solely by the sumac extract & did not contain contamination from the other species of bacteria present in sample(s).

Table 3: Sumac Extract preparation and characterization.

Extraction Method	Solvent Used	TPC (mg GAE/g)	TFC (mg QE/g)
Maceration	Ethanol	120.4 ± 3.2	98.7 ± 2.5
Maceration	Methanol	115.2 ± 4.1	95.3 ± 3.1

Comment 3: The method employed for preparing the sumac extract preserves the primary antifungal active ingredients of sumac extracts. Maximum efficacy of the extraction process for polyphenolics and flavonoids was achieved with continued use of ethanol and methanol as solvent for polyphenolic extraction, which was indicated by TPC and TFC values. Additional more refined analysis of individual layers using HPLC equipment further validates the antifungal properties of some polyphenolic constituents within the sumac extract. The bioactive coordinations of the total number of polyphenolics suggest that sumac extract could be an effective natural alternative to chemical antifungal products. Standardized extraction processes enable repeatable results that are required when developing a commercial product. The characterization of the sumac extract provides a solid foundation for evaluating its ability to inhibit both Aspergillus flavus growth and aflatoxin production.

Table 4: Inoculation conditions for A.flavus.

Treatment	Spore Concentration (CFU/mL)	Incubation Temperature (°C)	Humidity (%)
Control	1 x 10^5	27	85
Treated	1 x 10^5	27	85

Comment 4: Researchers ensure that their experiments maintain experimental reliability when they use standardized inoculation methods by reducing the amount of environmental variability present in each experiment. In order to provide an accurate determination of spore concentration (1 x 10^5 CFU/mL) to optimize fungal growth, the

spore concentration must be at this level for all experimental processes evaluating the inhibitory effects from the spore inoculum. Research evaluations of how sumac extract has an impact on fungal growth are also feasible, as the standard laboratory conditions of evaluating sumac extract on all of the experimental samples are maintained during testing and provide for reliable measurements within the experimental conditions.

Table 5: Treatment application and incubation time

Treatment	Sumac Extract Concentration (%)	Incubation Duration (Days)
Control	0	7/14
Sodium Benzoate	0.1	7/14
Sumac Extract	0.5	7/14
Sumac Extract	1	7/14
Sumac Extract	2	7/14

Comment 5: The antifungal efficiency evaluation benefits from different sumac extract concentration tests in this study. The recorded results over 7 to 14 days' supply data about antifungal performance both at the beginning of and throughout the testing period. The study incorporates sodium benzoate as a reference antifungal treatment to determine how effectively sumac extract performs against sodium benzoate. Scientists use research design techniques to understand how increased extract concentrations affect the rate of fungal inhibition as well as the formation of inhibition plateaus during fungal growth. Detailed research on sumac extract's dosage-effect relationship is crucial for understanding its impact on future food preservation methods.

Table 6: Fungal Growth Reduction (CFU count).

Treatment	CFU Reduction (%)
Control	0
Sodium Benzoate	68.4 ± 2.1
Sumac Extract (0.5%)	45.7 ± 3.2
Sumac Extract (1.0%)	72.5 ± 2.8
Sumac Extract (2.0%)	88.3 ± 2.5

Comment 6: *Increase in sumac extract concentrations results in increasing antifungal activity, thus enabling a further decrease in CFU's. 2% sumac extract showed between equal measurements of antifungal ability when compared to sodium benzoate, indicating its potential to serve as a viable organic alternative to sodium benzoate. Statistically valid testing enabled the ability to substantiate sumac's bioactive properties. Additionally, in preventing fungal contamination of stored food (through testing) the reduction of fungal growth provides a basis for the antifungal activity. In addition to the antifungal activity provided by these extracts, an extension of the current research is necessary to understand how long fungus will be exposed to sumac extract and if they are affected by this exposure over time. Based on the current research, sumac extract appears to present a viable option as an efficacy component of food safety antifungals.*

Table 7: Alfatoxin Inhibition (%)

Treatment	Aflatoxin Reduction (%)
Control	0
Sodium Benzoate	75.2 ± 3.1
Sumac Extract (0.5%)	51.3 ± 2.9

Sumac Extract (1.0%)	78.6 ± 3.4
Sumac Extract (2.0%)	91.5 ± 2.7

Comment 7: The most potent sumac extract treatment decreased aflatoxin production amounts by more than 90 percent. The observed results point to a two-step effect that combines fungal growth limitation with inhibiting aflatoxin bioformation. The observed findings demonstrate sumac's promising potential as a food safety solution that helps replace synthetic preservatives in traditional food systems. Public health benefits significantly from the capability of aflatoxin reduction because aflatoxins behave as carcinogens in the human body. Studies should analyze how stable sumac extract maintenance performs against microbial growth when used as a long-term food safety agent over prolonged storage times.

Table 8: Statistical Analysis Results

Treatment	Mean ± SD	p-value
Control	0.00± 0.00	-
Sodium Benzoate	71.8 ± 3.0	<0.05
Sumac Extract (0.5%)	48.5 ± 3.1	<0.05
Sumac Extract (1.0%)	75.6 ± 2.7	<0.05
Sumac Extract (2.0%)	89.9 ± 2.6	<0.01

Comment 8: According to the statistical analysis, there are higher concentrations of sumac extract and there are higher concentrations of sumac extract being produced. The results of this study confirm that these extracts have both anti- fungal properties and the ability to reduce aflatoxins. The means and standard deviations of the replicates in this study support the reliability of the findings. The p-values were less than 0.05 and confirm that there is a significant amount of antifungal activity as well as aflatoxin suppression by sumac extract. The results of this research suggest that sumac extracts could be an effective natural alternative to the use of synthetic food preservatives. There is a need for further studies to determine the best methods of utilizing sumac extracts and conducting safety and stability tests over time.

4. Discussion

According to the findings presented in this study, the use of Rhus coriaria extract has strong antifungal properties and inhibits the production of aflatoxins by Aspergillus flavus when applied to nut samples. The data indicates that when the sumac extract was applied at a ratio of 2.0%, the measured reduction in CFUs was 88.3% and that the extract was as effective as sodium benzoate in terms of the level of inhibition (68.4%). When applied at the highest

concentration of extract, aflatoxins produced by Aspergillus flavus were reduced by 91.5%. This suggests that sumac could be an effective plant-based alternative to existing chemical strategies for inhibiting the growth of fungi and meeting consumer demand for alternative methods for food preservation [6].

Plant-derived polyphenols have been demonstrated to inhibit the growth of fungi that can produce mycotoxins. Pomegranate peel extract in ethanol was able to reduce colony numbers of Aspergillus flavus fungus by 82% (Luo et al., 2018), which supports our research findings. Flavonoids and tannins contained in sumac have antifungal activity and have been identified as potential contributors by other researchers [7]. In contrast, cinnamon extract has shown 76% inhibition of aflatoxins [8], however, this effect was not

replicated in this study. The combination of several different polyphenols/flavonoids present in sumac extract demonstrates that the extract has significant antimicrobial activity based on its detrimental effects on fungal cells. Fungal cell membranes of Rhus coriaria extracts were damaged by each treatment, thus preventing the production of mycotoxins.

Sumac extract demonstrates antifungal characteristics mainly because of its high total phenolic content (TPC) and total flavonoid content (TFC). The study used HPLC analysis to verify the presence of antifungal polyphenols such as gallic acid, quercetin, and tannins, which have shown antifungal properties. The anti-fungal properties of the bioactive compounds present in the sumac extract are presumed to degrade the walls of the fungal cells and inhibit fundamental biosynthetic pathways responsible for producing mycotoxins just like grape seed extracts [9]. From the evidence put forward by Mamo et al. [10], it can be seen that natural antifungal compounds not only reduce the growth of fungi but also contribute towards food security because of a reduction in contamination from aflatoxins.

The statistical data proves the reliability of the observed antifungal properties. The statistical test performed using One-way ANOVA showed significant differences between groups, and Tukey's test proved that sumac extract at 2.0% is most effective.

There exists high probability that sumac extract will have numerous applications in the area of food processing industry. Due to its high levels of antifungal activity and ability to decrease exposure to aflatoxins, sumac extract presents itself as an environmentally friendly substitute for chemical preservatives. Further research should be conducted in order to optimize composition of sumac extract and assess its impact on food preservation when applied under storage conditions and its impact on the quality of taste in products [11]. [12]. It is important to investigate interactions between sumac extract and other natural antifungal components in order to enhance effectiveness of this technique. According to recent studies, interaction of plant-based antifungals enhances efficacy and minimizes chances of development of fungi resistance. Toxicity tests should be performed in order to dispel worries concerning safety of sumac extract for human consumption

5. Conclusions

According to studies, sumac extract contains potent antifungal properties and the ability to inhibit aflatoxin, making it a suitable alternative to synthetic preservatives. This evidence supports previous findings and indicates that sumac extract has superior antifungal activity compared to other natural antifungal agents. More research is needed by industry to investigate the potential for sumac extract to be utilized on a larger scale as an additive in food, with the need to develop optimal methods for extracting the active compounds from sumac and to conduct long-term safety assessments [13] [14] & [15].

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