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Antifungal Efficacy of the crude Alkaloid, Flavonoid, and Terpenoid of *Saussurea costus* (Falc.) Lipschitz Roots against *Aspergillus* species isolated from Rice Seeds

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Abstract

Background: Rice is an essential component of the diets of people all over the world, and it accounts for a sizeable amount of the calories that they consume. The world's food supply suffers huge economic losses due to fungal deterioration on rice seeds. The medicinal plant *Saussurea costus* contains bioactive chemicals and is well-known for its many therapeutic uses. There are a number of species of *Aspergillus* that are considered to be diseases in vegetables and fruits. One of these species is *Aspergillus niger*. The purpose of this current investigation was to investigate the impact of the crude Alkaloid, Flavonoid, and Terpenoid chemicals derived from *Saussurea costus'* roots on Aspergillus species Purified from Rice seeds obtained from various markets in Hillah, Iraq.

Methods: In vitro, antifungal activity against *Aspergillus* species was achieved using the food poisoning method. Three concentrations 5, 10, and 15mg/ml of each crude compound were prepared and compared to a positive control represented by Carbendazim500g/l and a negative control represented by 10% dimethyl sulfoxide.

Results: An objective of the current investigation was to manage the *Aspergillus* species growth identified in Rice seeds by utilizing secondary compounds derived from the roots of *Saussurea costus*. The study's data indicated that the extracts represented by active compounds such as Alkaloids, Flavonoids, and Terpenoids obtained from *Saussurea costus* roots exhibited a significant decrease in the of *Aspergillus* species growth, particularly at a concentration of 100mg/ml, in contrast to the negative control. This effect was equivalent to that of the positive control, Carbendazim 500g/l.

Conclusion: Finally, *Saussurea costus* roots have shown the most efficacy in controlling *Aspergillus* species, particularly through the presence of Alkaloid, Flavonoid, and Terpenoid chemicals at a concentration of 100mg/ml.

Introduction

Oryza sativa L., represents over 50% of an essential dietary constituent of the people around the world. It contributes approximately 55 to over 79% of the entire caloric eating for individuals residing in South and Southeast Asia, and Latin America, in other places, it is regarded as a valuable cash yield [1]. Rice is crucial for ensuring food security in Iraq and various other countries [2]. Molds that ruin food and feed result in significant global economic losses. Approximately 5 to 10% of the global food supply is lost as a result of fungal decay [3]. Over 100 distinct fungal species have been found on rice seeds thus far. However, the degree of these effects varies based on the timing of sample, location, and different types [4]. *Saussurea costus* (Falc.) Lipschitz, is a species of the family *Asteraceae* and is a species native to the Himalayas, specifically found at heights ranging from 2700 to 4000 meters in Lahul Valley of Himachal Pradesh, Garhwal of Uttarakhand and Kashmir, and has another name *Saussurea lappa* C.B. Clarke [5, 6]. *S. costus* is a renowned and significant medicinal plant extensively utilized in diverse indigenous medical systems to treat a range of conditions, including asthma, inflammatory illnesses, ulcers, and stomach problems. The primary phytoconstituents of this plant are sesquiterpene lactones. Various pharmacological experiments conducted in both laboratory and animal models have provided strong evidence of *S. costus*' capacity to display anti-inflammatory, antiulcer, anticancer, and hepatoprotective properties. These findings support the traditional applications of this plant. The plant contains dehydrocostus lactone, cynaropicrin, and costunolide which have been identified as possible bioactive compounds. Given the notable biological action of *S. costus* and its ingredients, it is fitting to explore their potential as a pharmaceutical treatment [7]. Fresh roots obtained from *S. costus* plant contain lactone, lappadilactone, dehydrocostus, cynaropicrin, and germacrenes compounds [8]. *Aspergillus* is among the top three fungal genera that have a substantial effect on food deterioration and the production of mycotoxins. The other two key genera are *Fusarium* and *Penicillium*. Among these, *Aspergillus niger* is the sole public species that poses a solemn threat as a pathogen in some vegetables and fruits [9]. Instances of mycotoxin production by *A. niger* strains are infrequent [10]. *Aspergillus* species are capable of producing significant mycotoxins, specifically aflatoxins which are generated by *A. flavus* and similar species, and ochratoxin A, which is formed by *A. ochraceus* and associated species, as well as *A. carbonarius* [11]. The objective of this investigation was to manage the growth of *Aspergillus* spp. found in Rice seeds by employing secondary metabolites derived from the roots of *S. costus*.

Methods

Plant active part: Roots belonging to *S. costus* had been procured from indigenous markets, and thereafter recognized based on their characteristics [12], The roots of this plant were cleansed, desiccated, and stored under [13], (Fig: 1).

Figure 1: *Saussurea costus* (Falc.) Lipschitz roots.

The crude alkaloid components had been extracted according to the method reported in [14].

The crude flavonoid components had been extracted according to the method reported in [15].

The Crude Terpenoid components had been extracted according to the method reported in [16]. A 100 mg/ml stock solution of Alkaloid, Flavonoid, and Terpenoid was made in a 10% Dimethyl Sulfoxide (DMSO) solution. The solution was then sterilized using a Millipore filter with a pore size of 0.22µm and stored at -20°C pending it was ready for use [17].

Rice Seed collection: rice seeds were gathered from various parts of indigenous shops in the area of Babil, namely in Hillah-Iraq, 2022.

Isolation and diagnosis: To separate the *Aspergillus* fungi, a total of one-hundred seeds were haphazardly selected from each sample. The sterilization process involved treating it with a 2% solution of sodium chlorate for 2 minutes. Subsequently, it was rinsed

twice with sterile distilled water to eliminate any remaining sterile substances. Finally, it was dried using sterile filter paper. The specimen was carefully transferred using sterilized forceps onto 9cm Petri dishes that contained 20 ml of preprepared Dextrose Agar of Potato (PDA) supplemented with 50mg/l of chloramphenicol to inhibit bacterial growing [18], each dish was seeded with 5 seeds, and each sample was replicated three times. The dishes had been incubated at 25°C for 5 to 7days. The fungi that were present in the seeds were subsequently isolated using secondary cultures in order to be identified. The isolated were then identified using the keys in [19, 20]. The isolates were stored in aseptic glass containers filled with Nutrient Agar media. The containers' glass had been kept at 25°C for a duration of 7days, thereafter deposited in a fridge-freezer 4°C pending they were utilized.

Antifungal assay: The PDA medium had been prepared and sterilized. Then, a volume of (1, 2, 3ml) of each plant extract had been placed in the center of the Petri dishes. The volume had been completed to 20ml with PDA medium to achieve the desired final volume concentrations (5, 10, and 15 mg/ml) of the secondary metabolites under study. Later the medium hardened entirely, a disc about of 5mm in diameter of a 7-day-old of the test fungus had been placed in the center of the Petri plates under sterile conditions. The plates were then incubated at a temperature of 25±2C˚ for 7days. Simultaneously, 0. 02ml of an antibiotic solution had been added to each assay plate to check for bacterial contamination, as recommended by the suggested protocol [21]. The positive control utilized in the experiment was Carbendazim, a fungicide with a concentration of 500g/l. The negative control, on the other hand, was dimethyl sulfoxide with a concentration of 10%. Data was documented on the 7th day. The measurement of the colony's diameter had been expressed in millimeters. A control had been used as a medium without extract. Three replicates had been maintained for each treatment. The toxicity of the f extracts had been determined by calculating the percentage of inhibition in mycelial growth, by means of the specified formulation [22].

$$\text{Percent Inhibition\%} = [dc - dt / dc] \times 10^2$$

dc=The mean increment in mycelial diameter colony under normal conditions.

dt=The mean increment in mycelial diameter colony within the experimental treatment.

Statistical analysis: The data for treatments had been obtained from 3 replicates. The data underwent an analysis of variance by means of the SPSS, 20.0 version. A design of completely randomized had been employed, the least significant difference (L.S.D.), test had been conducted at a significance level of Probability ⩽ 0.05.

Results

Alkaloid antifungal crude compounds extracted from *S. costus* roots in contradiction of *Aspergillus* spp. isolated from seeds of Rice had been assessed in Table, 1. The findings demonstrated that Alkaloid secondary products derived from *S. costus* roots exhibited a significant decline at P⩽ 0.05 of *Aspergillus* spp. growth and the same effect of the positive control. Antifungal action was applied at (5.0, 10.0, and 15.0) mg/ml. mycelial reduction fluctuating from 66.0% in 5.0 mg/ml, 71.6% in 10.0 mg/ ml, and 100% in 15.0 mg/ml, (Figure 2: A, B), compared with negative control treatment represented by 10% DMSO and positive fungicide Carbendazim 500g/l control (Figure 2: C) where redaction proportion was 0.00% for control treatment and 100% for fungicide Carbendazim 500g/l positive control treatment, (Figure 2: D).

Concentrations	Alkaloid Antifungal Reduction%
N. Control	0.0±0.00
5.0mg/ml	66.0±1.00
10.0mg/ml	71.0.6±0.57
15.0mg/ml	100.0±0.00
P. Control	100.0± 0.00
L.S. D.	0.939

Table 1: Alkaloid Antifungal of *S. Costus* root against *Aspergillus* species.

Within the same context, the crude Flavonoid compounds exhibited a 62.3% suppression of mycelial growth at a concentration of 5.0 mg/ml, and complete inhibition at concentrations of 10.0 mg/ml and 15.0 mg/ml, as indicated in table 2. Therefore, it exhibited substantial differences in comparison to the control treatment, as well as producing a similar effect to the positive control, (Figure 2: E, F).

Concentrations	Flavonoid Antifungal Reduction %
N. Control	0.0±0.00
5.0 mg/ml	62.3±0.57
10.0 mg/ml	100.0±0.00
15.0 mg/ml	100.0±0.00
P. Control	100.0±0.00
L.S. D.	0.470

Table 2: Activity of Flavonoid Antifungal of *S. Costus* root against *Aspergillus* species.

The crude Terpenoid compounds exhibited noteworthy efficacy at three doses (5.0, 10.0, and 15.0 mg/ml), in comparison to the negative control. Additionally, they demonstrated a similar impact as the positive control at concentrations of 10.0 and 15.0 mg/ml, (table 3). The inhibitory action exhibited a percentage of 34.1% at a concentration of 5.0 mg/ml, while reaching 100% at concentrations of 10.0 and 15.0

mg/ml, respectively, (Figure 2: G, H). Finally, Secondary metabolite products, including Alkaloid products, Flavonoid products, and Terpenoid products, isolated from *S. Costus* roots exhibit similar effects to the positive fungicide Carbendazim 500g/l control.

Concentrations	Terpenoid Antifungal Reduction %
N. Control	0±0.00
5.0mg/ml	34.1±1.04
10.0mg/ml	100.0±1.00
15.0mg/ml	100.0±0.00
P. Control	100.0±0.00
L.S. D.	1.174

Table 3: Terpenoid Antifungal *S. Costus* root against *Aspergillus* species.

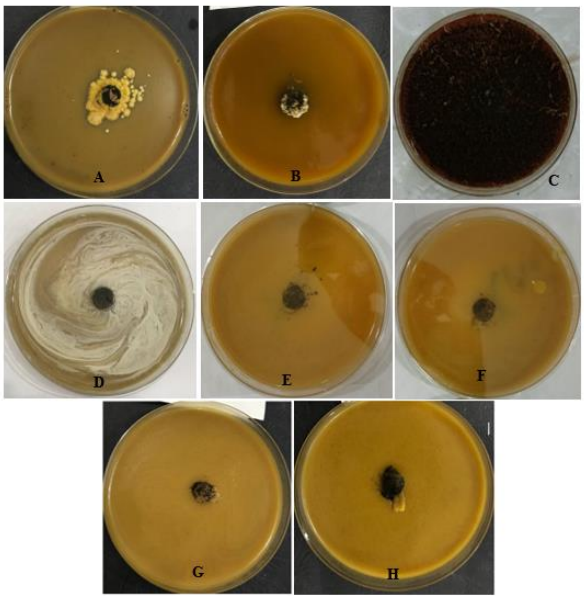

Figure 2: Effect of antifungal efficacy in growth of *Aspergillus* spp. A- Alkaloid antifungal at 10.0mg/ml; B- Alkaloid antifungal at 15.0mg/ml; C- growth of *Aspergillus* spp. in control treatment; D- growth of *Aspergillus* spp. in the fungicide Carbendazim 500g/l treatment; E- Flavonoid antifungal at 10.0mg/ml; F- Flavonoid antifungal at 15.0mg/ml; G- Terpenoid antifungal at 10.0mg/ml; H- Terpenoid antifungal at 15.0mg/ml.

Discussion

Medical plants are regarded as a crucial resource for the treatment and prevention of numerous types of diseases [23]. Every plant has numerous essential components that have potential use in the field of medicine and can contribute to the creation of various pharmaceuticals [24]. The current investigation demonstrated that chemical secondary compounds, including Alkaloids, Flavonoids, and Terpenoids, derived from *S. costus* roots exhibited anti-fungal properties counter to *Aspergillus* spp. obtained from rice seeds collected from several local markets of Hillah city located in Iraq. Chemical secondary compounds derived from several active parts of different medicinal plants such as *Lactuca serriola* leaves, *Lepidium sativum* leaves, *Myrtus Communis* leaves, *Cassia senna* leaves, *Ricinus communis* leaves, *Cassia didymobotrya* leaves, *Melia azedarach* leaves, *Dianthus caryophyllus* flower buds, and *Salvia hispanica* seeds, exhibit antibacterial properties capable of suppressing various pathogenic microorganisms obtained from diverse clinical samples [25-33]. Hussein *et. al.* [34] it has been shown that phytochemical substances isolated from the unicellular primitive plant *Chlorella vulgaris* have the capacity to counteract harmful bacteria by acting as antibacterial agents. Kamal *et. al.* [35] Used *Hibiscus sabdarifa* extracts for controlling pathogenic microorganisms such as *E. coli* and *Proteus* sp. Kamal *et. al.* [36] Employed *Ficus carica* for inhibiting *Pseudomonas aeruginosa* and *Escherichia coli* pathogenic bacteria. AL-Masoodi *et. al.* [37] Utilized substances derived from *Curcuma longa* and *Boswellia carteri* to manage *Fusarium* spp. obtained from corn seeds. Referring to[38, 39] Employed terpenoids derived from seeds of *Carthamin tinctorius* and flavonoids obtained from *M. Communis* leaves to combat *Aspergillus* spp. identified from preserved seeds of medicinal plant. Secondary compounds found in *M. Communis* leaves, including alkaloids and flavonoid compounds, are considered valuable for their ability to inhibit pathogenic microorganisms that are isolated from hemodialysis fluid specimens [40]. Radhi *et. al.* [41] Employed extracts from *Callistemon viminalis* leaves to manage isolates of Urinary Tract Infections. *Allium sativum* is considered a reliable means of suppressing some bacterial species that have been isolated from people infected with the Corona Virus [42]. The flower buds of *D. caryophyllus* contain secondary metabolite chemicals, specifically terpenoids and flavonoids, which have potent antifungal properties against *Candida* species [43]. *S. costus* was regarded as a therapeutically significant plant, the numerous compounds extracted from it have remedial properties [12]. The roots of *S. costus* contain alkaloids and terpenoids that exhibit potent antifungal properties against *Candida* species [44]. Several chemicals were extracted from the roots of *S. costus* and evaluated against four species of *Aspergillus* such as *A. niger, A. ochraceus, A. versicolor, A. flavus,* and two species of *Penicillium* such as *P. ochrochloron, P. funiculosum,* and other species such as *Cladosporium cladosporioides, Alternaria,* and *Trichoderma viride.* The chemical exhibited anti-fungal properties ranging from temperate to strong [45]. The polyphenol content of *S. costus* was found to be highest in the ethanol and ethyl acetate extracts, n-butanol extract came next, while n-hexane extract came last, these compounds exhibit antibacterial and antifungal properties against a wide range of microorganisms [46]. *Saussurea lappa* had the most potent antifungal action against *A. flavus*,

with *Trapa natans* and *Mangifera indica* showing lesser efficacy [47]. The methanolic extract of *S. costus* roots is abundant in many components including flavonoids, terpenoids, alkaloids, phenols, polyphenols, tannins, resins, coumarins, quinines, steroids, and cardiac glycosides exhibited antifungal properties against *A. niger* strain (ATCC6275), [48]. Conversely, the mechanism by which Alkaloids exert their antifungal effects is typically pleiotropic, involving inhibition of protein synthesis and intercalation into fungal DNA, or by improving the production of fungal inhibitors. [49]. Terpenoids decreased the number of mitochondria, hence altering the levels of production of ATP and reactive oxygen species. Additionally, it has been found that triterpenoids have greater antifungal action in comparison to tetraterpenoids [50]. Secondary products such as Terpenoid and flavonoid exert their own effects by destabilizing microbial membrane components [51]. Flavonoids commonly hinder the growth of fungi by diverse ways, such as disrupting the plasma membrane, causing dysfunction in the mitochondria, and inhibiting processes including cell wall production, cell division, RNA, and protein synthesis, as well as the efflux-mediated pumping system. [52]. Therapeutic herbs exhibit anti-fungal properties through numerous methods. They disrupt the cell membrane, leading to an injury of membrane integrity. They also hinder DNA transcription and decrease the number of fungal cells. Additionally, they block the action of fungal antioxidant enzymes and prevent the creation of fungal biofilms [53, 54]. Finally, the anti-fungal activity of *S. costus* roots may be attributed to alkaloids, flavonoids, and terpenoids. These compounds could affect proteins and DNA synthesis, as well as impair membrane permeability and disturb metabolic activity.

The roots of *S. costus* include alkaloids, flavonoids, and terpenoids that exhibit potent antifungal properties against *Aspergillus* spp. that exist in rice seeds.

Author Contributions

Fatima S. Al-Nafie: study design, Hussein J. Hussein: Refinement of study design and supervision, Abeer Fawzi Al-Rubaye: Refinement of study design and supervision.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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