Antifungal and Antioxidant Activities of Methanol Extract of Chitin, Chitosan and Shrimp Shell Waste

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ABSTRACT

The present investigation aimed to evaluate chitosan, shrimp shell waste and chitin against the fungus activity. Shrimp shell methanolic extract showed stronger inhibitory effects than chitin. Thus, the inhibition zone reached (19.5 mm) for Asprigellus fumigatus and reached (16.5 mm) against both A.flavus and A.niger, while the chitosan extract showed the lowest effectiveness. The natural treatments for Candida albicants were not effective. In contrast, Nesoral as chemical product recorded the maximum inhibitory activity for all tested fungi. The antioxidant properties were observed through total polyphenolic compound. The results indicated that chitin, chitosan and shrimp shell exhibited the highest antioxidant properties. The present finding revealed that the tested Chitin, Chitosan and Shrimp shell waste are promised materials from natural sources as antifungal agents.

Key Words: Antifungal Activity, Chitin, Chitosan, Shrimp Shell Waste

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INTRODUCTION

Chitin is a natural polysaccharide produced by many living organisms and a crystalline structure and it constitutes a network of organized fiber [1]. Chitosan, a cationic polysaccharide with a high molecular weight, linear polymer, comprises -1,4- linked glucosamine (GlcN) with various amounts of N- acetylated GlcN residues. It is usually formed via the alkaline deactivation of chitin extracted from a profuse attention [2]. Chitin is widely disseminated in environment, mostly as a structural polysaccharide present in fungal cell membrane, the outer shells of crustaceans, nematodes and the exoskeletons of arthropods. About 75 % of the total weight of shellfish, such as crab, krill, and shrimp is considered waste. The dry weight of waste contains about 20-58% chitin [3]. The costal countries are famous for production of large quantities of shell fish sharing massively to the food delicacies. The meat of prawns, lobsters and shrimp are extracted while the remaining
parts of these fishes like the shell and the head are considered as wastes which has become a serious environmental concern [4]. Chitosan has been indicated for the preservation of foods [5, 6], and juices [7], during withdrawal of chitosan from waste of Prawn shell, many testes are subjected to estimate its availability for commercial purposes [8] and other materials from microbial deterioration acting with various kinds of microbes particularly as antibacterial action [9-12], yeast and fungi [13-25]. Recently more of attention was paid for chitosan, chitin and its byproducts and its possible activity or application as antibacterial or antifungal or anti-yeast activity. Many authors [26-32], reported that chitosan and chitin are safe material to induce resistance against soil-borne and seed borne fungi and induced suppressive action on pathogenic fungal growth. Earlier studies indicated that

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Chitosan, a biopolymer of glucosamine derived from chitin is a biopolymer with antioxidant properties. The pharmacological studies on chitosan and chitin showed that it can lower the level of cholesterol in the blood, also it was found that chitosan oligosaccharides had an effect on hyperglycemic animals through reducing the glucose concentration in the blood. Some researchers found that chitosan and chitin are very important because they possess nearly high contents of nitrogen in comparison with synthetic cellulose [9]. Marine invertebrates have a defensive mechanism against most of pathogens which depend on innate immune mechanisms which comprise humoral and cellular responses. In marine invertebrates, both the blood cells and plasma carry the humoral immune system which protect the body form invading microorganisms, whereas, the cellular immunity depending mainly on cell defense mechanism, include nodule formation, encapsulation of foreign bodies and finally phagocytosis [10]. The current work was aimed to study the antifungal activity of shell fish by products such as chitosan, chitin and shrimp shell.

MATERIALS AND METHODS

Collection of materials

Samples of Chitin, Chitosan and Shrimp shells were collected during May, 2014 from Jeddah city in west region of Saudi Arabia. Shrimp shells were obtained from the fish market and all shells were from a single species of shrimp, while the chitosan and Chitin collected from factory.

Samples preparation

The shrimp shells were washed by warm water to remove soluble organics, adherent proteins and other impurities, then boiled in water for 1 h to remove the tissue, followed by drying in an oven (Prolabo, model Volca MC18, French) at 160 °C for 2 h to break down the crystalline structure of chitin and to make them more brittle [33]. Finally, the dried shells were ground as a powder using a standard grinder (Model KU-2, PredomMesko, SkarzyskoKam., Poland).

Extract preparation

According to Boeru and Derevici method (1978) [34] briefly, ten grams of dried samples extraction was done by adding 100 ml of methanol. The solutions were collected after filtration, evaporated under reduced pressure at 40°C until dryness, the ended diluted by dimethyl sulfoxide (DMSO) and stored at 20°C for analysis.

Microorganisms

The test organisms used in this study (Aspergillusfumigatus, Aspergillusniger, Aspergillusflavus, and the yeast Candida albicans) were obtained from King Fahed Hospital in Jeddah. Sterile Sabouraud dextrose agar (SDA) was poured in Petri plates for fungi cultivation. To prepare the inoculums, a portion of tested fungus was inoculated into 10 ml sterile water (saline solution). 1 ml of the suspension was transferred to a flask containing 50 ml sterilized medium (45°C) giving 1 ×106 fungus per ml. For solidification, the medium was shaken well and poured into Petri dishes.

Antifungal test

According to [35], the well-cut diffusion technique was tested. Wells were cut from the plate by using a sterile cork borer (0.5 cm). Methanol extract of chitin, chitosan and shrimp was introduced into each well, DMSO was used as a negative control and the plates were maintained at 4°C for 2 h. Later, the plates were incubated for 2-4days at 27°C. The diameter of the growth inhibition holes was measured in millimeters. All assays were performed in triplicate [36].

Estimation of Total Phenolics (TPC)

The total phenolic content was determined by using the FolinCiocalteu assay [9]. An aliquot (1 ml) of extracts or standard solution of Gallic acid (0.75ml of 20g/100ml sodium carbonate) was added to 25 ml of volumetric flask, containing 3 ml of distilled water. Reagent blank using distilled water prepared 0. 5 ml of Folin-Ciocalteu phenol reagent was added to the mixture and shaken. After incubation for 20 minutes in water bath at 40°C, the absorbance against the reagent blank was determined at 765 nm with an UV-Visible spectrophotometer. Total phenolics content was expressed as mg Gallic acid Equivalents (GAE). The experiments were performed in triplicates.

Statistical analysis

Data were analyzed in three replicates for each treatment and as a result three determinations were conducted. With the help of SPSS, standard error and means of variable were calculated to authenticate the significant differences between both the pathogenic extract types and micro-organisms.

RESULTS AND DISCUSSION

Data presents in (Table1 and Fig. 1) showed that the shrimp shell, chitin and chitosan methanolic extract were tested for their antifungal activities against four tested pathogenic fungi including Aspergillums fumigates, Aspergillumsflavus, Aspergillumsniger, and C. albicanis. As appeared in the results shrim shell, methanolic extract showed the strongest inhibitory effects than chitin. Thus, the inhibition zone reached (19.5 mm) for Aspergillumsfumigatus and (16.5 mm) for Aspergillumsflavus and Aspergillumsniger, respectively. This followed by chitin methanol extract which produced inhibition zone reached (16.5, 15.0 and 14.5 mm) for the

following fungi A. flavus, A. fumigatus, and A. niger respectively. whereas, the chitosan extract showed the lowest effects against the fungi compared with other extracts, the inhibition zone reached (14mm) against Aspergillumsfumigatus; (12 mm) against A. flavus and A. niger, respectively. In contrast, the present data showed that the highest antifungal activities of Nesoral as chemical product positive against the tested fungi compared with natural treatment.

Table 1. The antagonistic effect of methanol extract of Shrimp shell, chitin and chitosan against some pathogenic fungi expressed by inhibition zones (mm).

Treatment	A.fumigatus	A.flavus	A.niger	C.albicanis
Shrimp	19.5	16.5	16.5	0
Chitin	16.5	15	14.5	0
Chitosan	14	12	12	0
nesoral	30	38	36	35

Our results coincided with some investigators [37] who reported that the antifungal compound of methanol extracted of chitosan show elevated inhibitory area in comparison with that extracted with ethyl acetate. Some workers reported that addition of chitosan to T. harzianumculture media, increased significantly the suppressive activity for F. oxysporum in vitro and can be used as biological control for controlling of Fusarium wily disease in some vegetables as in tomato plant [38]. The influence of chitosan with fractions of acetylation and polymerization supplementation on some fungi activity was clear, where it induces complete suppression in the growth of B. cinerea, R. stolonifer, and A. alternate fungi, while it retards the growth of P. expansum fungus [39]. In agreement with our result, [40] reported that antifungal activity of Aliette, Antracol, Defenaconazole Aalone was lower than treatment by mixing with chitin on some fungal microorganisms infection Chili Seeds (Aspergillusflavus, A.niger, Alternariaalternata, A. solani, Fusariumoxysporum, F. solani, Rhizoctoniasolani, and Pythium spp.)

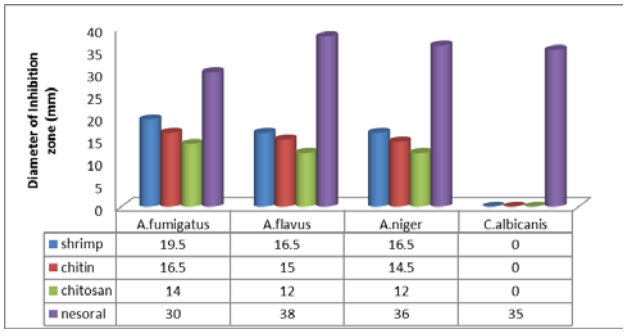

Fig 1. The antagonistic effect of Shrimp, chitin and chitosan against some pathogenic fungi expressed by inhibition zones (mm).

The antimicrobial influence of the extracts of chitosan, shrimp shell and chitin were showed to have very different effectiveness on fungi, some were more resistant and others were highly sensitive, so three treatments seemed not to have anti-fungal influence particularly against Candida albicanis. This is supported by many authors [41, 42] that the tunicamycin and Benzoylphenylureas had a weak suppressive effect of chitin on fungi, whereas the polyoxins and nucleotide analogous nikkomycins had the strongest effects. The pharmacological action of chitosan on the fungi considered fungi static which suppresses the growth of fungi not having a lethal activity on fungi with a possiblity to associate regulatory changes in the fungus and host [43].

The data of fungi static support the hypothesis that some of possible mechanisms have been proposed to illustrate the activity of chitosan against microorganism, they postulated that the positive charge conferred by protonation of free amino groups at acidic pH., where the polycationic chitosan can join partially with the cell membrane of the fungus which carry negative charge (i.e. phospholipids, proteins), thus interfere with the normal growth and metabolism of the fungal cells [39, 44]. Roller and Covill [7] found that the contents of chitosan of amino groups can interact with anionic groups which are present in the cell membrane of the yeast leading to formation of impermeable biofilm layer around the cell, so the chitosan might play a role as membrane barrier (e.g anionic groups) and accordingly, diminishing the activity and growth of microorganisms [39]. Other investigators said that the mechanism of action of chitosan as anti-microbial material through suppression of mRNA, which leads to suppression in the synthesis of the arrival of chitosan inside the nucleus of the microorganism and its join with DNA of the microorganism [12, 45]. Chitosan was found to be more effective in inhibiting radial growth, spore germination and germ tube elongation [17]. Microscopic observation showed that chitosan oligomers can suppress the enzymes responsible for the growth of the fungus via penetration of fungal hyphae [46].

The present results were in accordance with Sanivada and Challa [47] who observed that fungistatic effect of the tested 23 strains of entomopathogenic fungi, where it was found that a positive correlation was recorded between release of chitinolytic enzymes (chitinase) and antimycolytic activity when chitin was supplemented to the culture medium against pathogen Colletotrichumfalcatum causing red rot of sugarcane Chitinolytic enzymes considered as important biocontrol agents against soil borne pathogens because they have the ability to degrade walls of fungal cell [48]. The shrimp shell waste was a source of chitinase enzyme which was produced from Bacillus licheniformis in the form of nitrogen and carbon resource showing higher antifungal activity against the tasted phytofungal pathogens

(Aspergillus sp., Fusariumsolani, Fusariumoxysporum and Rhizoctoniasolani). Chitosan could be a good source of drugs that may be used against fungal infection [49].

Several researches are postulated that antioxidant properties of Phenolic compound are mostly found in Shrimp, chitin and chitosan. It has been reported that polyphenols of marine sea weeds extracts, showed antioxidant activity [41]. Figure 2 reveals the content of total phenols (TPC) of marine Shrimp, chitin and chitosan. The data demonstrated that, the highest values of content of total phenols were appeared in Shrimp 38 mg/gdw, chitosan 64 mg/gdw and chitin66 mg/gdw respectively. A significant contribution of an antioxidant property has been found in phenolic materials present in the Shrimp, chitin and chitosan according to the TPC. Furthermore, some studies did not found a negative association between TPC and antioxidant capacity [41].

However, adverse conditions show existence of phenolic compounds in Shrimp, chitin and chitosan associated with their protective mechanisms. During the hot climate in early stage of the growth, higher amount of phenolic compounds is produced in order to prevent the photo oxidative damage and sea grazers, respectively [41]. The mechanism of phenolic compound as an antioxidant relies on the structure of aromatic rings that attached to the hydroxyl groups [45]. The hydrogen atom of the hydroxyl group will be donated to the unstable free radicals and thus terminating the oxidative activity. However, negative correlation between total phenolic contents and antioxidant capacity did exist in some studies [46].

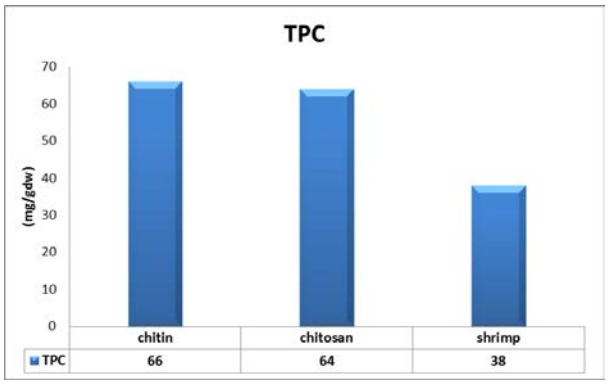

Fig 2. Total phenolic content (TPC) of Shrimp, chitin and chitosan

However, some studies suggested that there is a positive correlation between the scavenging activity and total phenolic content of the extracts that showed the presence of phenolic contents within the Shrimp, chitin and chitosan which might be the major contributors to the antioxidant activity of Chitosan.

Several studies have observed the role of phenolic contents in relation to the antioxidant activity [42]. The compounds belong to phenolic, flavonoid, tanin, and alkaloid groups, and the compounds with many sulfide groups. In addition, a series of polyphenolic compounds and related phenolic compounds such as epigallocathecin, cathechol, caffeic acid, myricetin and hespedirin have been isolated from the chitosan [43].

The antioxidant property of chitin was found less when compared to the chitosan. The total phenolic content for the shrimp shell extracted of chitin and chitosan showed the antioxidant activity contents whereas maximum activity of (TPC) was found in the marine crab chitin than the chitosan [18].

CONCLUSIONS

The present study concluded that methanol extract of shrimp shell recorded the maximum inhibitory activity than chitin against three pathogenic fungi A.fumigatus, A.flavus, A.niger; whereas, the chitosan extract showed a lowest effectiveness. Three natural treatments could not produce any antimicrobial activity against Candida albicanis. However, the antifungal activities of Nesoral as chemical product positive showed the strongest inhibitory effect against all the tested fungi compared with other natural treatments.

Finally, it can be mentioned that methanol extract of shrimp shell, chitin and chitosan showed promising activity against the tested pathogens. Hence, this treatment is valuable for realizing of new bioactive compounds from natural sources which may enhance the discovery and development of new drugs. Our study also, pointed to the possibility of using of chitosan as a natural source of antifungal compounds.

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