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Evaluation of Crude Sesquiterpenoid Extract of *Ganoderma reissi* as a Natural Food Preservative

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KEYWORDS

Food preservative, *Ganoderma reissi*, Sesquiterpenoid

A B S T R A C T

Ganoderma is a popular remedial mushroom, which has been used in the traditional medicine for the prevention or treatment of a variety of diseases. In the present study we evaluated the inhibitory effect of sesquiterpenoid extract of *Ganoderma reissi* on fungal and bacterial growth in different media. Microbial growth inhibition by sesquiterpenoid extract of *Ganoderma reissi* was evaluated in the media with natural ingredient such as milk agar (MA), tomato juice agar (TJA), wheat flour agar (WFA) and pine apple juice agar (PAJA) with or without adding Ganoderma sesquiterpenoid extract, sodium benzoate alone or Ganoderma sesquiterpenoid extract along with sodium benzoate. The pH of the media was adjusted to 6. Three strains of bacteria viz. *Bacillus subtilis*, *Eschericea coli*, Staphylococcus *aureus*and one strain of fungus i.e. *spergillus niger* were used. The bacterial strains were maintained on nutrient agar medium while *A. niger* on Sabouro-dextrose-agar. The sesquiterpenoid extract of *Ganoderma* at 0.2% concentration significantly inhibited the growth of all microorganism on natural media ($P<0.01$). These results were comparable with inhibitory effect of sodium benzoate at 0.1%, and *Ganoderma* extract 0.1% plus sodium benzoate 0.05%. The sodium benzoate at 0.2 % concentration completely inhibited the growth of all microorganisms. Similarly combination of *Ganoderma* extract (0.1%) and sodium benzoate (0.05%)] fully inhibited the growth of all microorganisms in all media ($P<0.01$). Considering the findings of this study, and considering the side effects of synthetic preservative, crude sesquiterpenoid extract of *Ganoderma reissi* might be used as an appropriate food preservative.

Introduction

The strategies used to prevent food spoilage include use of microbial growth inhibitors, destruction of microbial cells and mechanical removal of microorganisms from the food. The growth of microorganisms may also be inhibited by

chilling, freezing, water activity reduction, nutrient restriction, acidification, and modification of packaging atmosphere, fermentation as well as through addition of anti-microbial compounds (1). Food anti-microbial compounds are the chemicals added to or present in food that check microbial growth or kill microorganisms. The major targets are the microorganisms that cause food poisoning and food spoilage. Food anti-microbial compounds are used alone or often in combination with other food preservatives.

These compounds are sometimes referred to as food preservatives. However, food preservatives include not only anti-microbial compounds but also antibrowning agent like citric acid and antioxidant like butylated hydroxyanisole.

It has been estimated that about 37.5 million Kgs of preservatives have been consumed in the United States alone in 1991 and 47.3 million kg in 2000. Worldwide use of traditional food preservatives is expected to increase by 4.1 % every year. Commonly used traditional preservatives include propionates, sorbates, and benzoates (1). Most food preservatives are either bacteriostatic or fungistatic. However they fail to preserve the food for longer duration. Aspergillus is one of the most common contaminant in the food and it is estimated that almost 50 species of Aspergillus are capable of producing many toxic metabolites most commonly 'Aflatoxins' (2). It is produced by species like A. flavus, A. parasiticus and A. nomicus, while 'ochratoxin A' produced by A. ochraceus and related species, 'sterigmatocystin' produced by A. versicolor and Emericella spp., 'cyclopiazonic acid' by A. flavus and A. tamarii (2).

Several studies have confirmed anti-microbial and preservative action of spices and essential oils(3,4). Rocken observed that in the sourdough, antifungal activity was strictly related to acetic acid production(4). More recently, the ability of sourdough lactic acid bacteria to inhibit mold growth was studied, which led to the identification of a strain of Lactobacillus plantarum 21B whose culture filtrate showed antifungal activity.

Phenyllactic acid was shown to be one of the major compounds occurring in the culture together with lactic acid and acetic acid (5). There are some reports of utilizing the plant extract as fungal inhibitors. Mahmound reported that 1000 ppm of different essential oil constituents, namely geraniol and thymol, completely inhibited fungal growth (6). Later Paster et al. (1995) found thymol, the main component of thyme essential oil, to be active against Aspergillus spp. when it was added to the medium (3).

Considering the anti-microbial effects of Ganoderma species, the current study was conducted to evaluate the crude sesquiterpanoid extract of Ganoderma reissi as food preservative.

Materials and methods

Sample Collection

The samples were collected from different parts of Mazandaran province (North of Iran), brought to laboratory and air-dried in Department of microbiology, Yasouj University of Medical Sciences, while the remaining part was ground and maintained in airtight plastic bag for further use.

Identification of Samples

The *Ganoderma* samples were identified using keys and morphological characters mentioned by Steyaert and Ryvarden (5, 6).The samples identified by Dr.Fourutan that he is expert man in plant mycology and he works in agriculture research center of mazandaran province in Iran.

Extraction of Sesquiterpenoid

One hundred grams of *Ganoderma* powder was suspended in 2 liter of chloroform (×2) overnight with initial warming. The filtrates were combined and evaporated under vacuum (Medica Instrument MFG.Co., India). The residue was collected and dissolved in 500 ml of aqueous lead acetate (4% w/v) and 500 ml of 95% ethanol (1:1 v/v). The resultant solution was evaporated to dryness under vacuum. The obtained residue (3.1 g) was dissolve in methanol and used for further works.

Microorganisms: Three strains of bacteria viz. *B. subtilis* (NCIM2010), *E. coli* (MTCC, 724) *S. aureus* (HAL, 2079) and one strain of fungus i.e. *A. niger* (YMU) were used in this study. The bacterial strains were maintained on nutrient agar medium while *A. niger* on Sabouro's dextraose agar. Table 1 shows the strain and sources of the microorganisms used in this study.

Table.1 Microorganisms used in this study

Culture	Strain	Source
Bacillus subtilis(Bs.)	NCIM2010	NCL
Escherichia coli(Ec.)	MTCC724	IMTECH
Staphylococcus aureus(Sa.)	HAL2079	NCL
Aspergillus niger(An.)	YMU	YMU

Evaluating the preservative activity

For the evaluation of fungal growth inhibition by sesquiterpenoid extract of Ganoderma, the media with natural ingredient such as milk agar (MA), tomato juice agar (TJA), wheat flour agar (WFA) and pineapple juice agar (PAJA) were prepared with or without adding Ganoderma sesquiterpenoid extract, sodium benzoate alone or Ganoderma sesquiterpenoid extract together with sodium benzoate. Table 2 shows the ingredients of each media used in this study. (Table2).

Evaluating of growth rate

Five micro liter of microbial suspension was inoculated at the center of respective plates. The plates were tightly sealed with Para film and incubated for 10 days at 28 °C. Diameter of the colonies was measured after incubation period and percent of growth inhibition relative to that on control media was calculated.(figure 1,2,3,4)

Determination of the MIC

The minimum inhibitory concentration (MIC) of sesquiterpenoid extract was determined by E-test (7). Microbial suspensions were prepared in sterile saline and adjusted to a density of 106 spore /cell ml-1, corresponding to 68 to 82 % transmittance at 530 nm (8). The plates were inoculated by dipping a sterile cotton swab into the cell suspension and spreading it across the surface of the agar. The plates were dried at ambient temperature for 15 min. before keeping the filter paper discs. A total of 5 sterile discs were kept on the surface of agar by maintaining a distance of 5 mm between two successive disks. The *Ganoderma* extracts were serially diluted and 10µl of each dilution was impregnated

separately in each disc. The plates were incubated at 28 ºC (72 hours) for fungi, and 37ºC (24 hours) for bacteria. The MIC was recorded for each dilution by measuring a clear zone around the disk ignoring sparse subsurface hyphae at the margins. Microcolonies within the ellipse were ignored (8).

Table.2 Composition of the natural media:

Recipe		Milk Agar (MA)	Tomato juice agar (TJA)	Wheat flour agar (WFA)	Pine apple juice agar (PAJA)
I)	**Cow milk**	1000 ml	-	-	-
	Tomatoes	-	1000 ml	-	-
	Pine Apple	-	-	-	1000 ml
	Wheat flour	-	-	20 gm	-
	Distilled water	-	-	1000 ml	-
	Agar-agar	25gm	25gm	25 gm	25 gm
II)	**MA**	MA+Ga	TJA+Ga	WFA+Ga	PAJA+Ga
	TJA	100ml	-	-	-
	WFA	-	100ml		-
	PAJA	-	-	100ml	-
	Ganoderma extract	-	-	-	100ml
	(Ga)	0.1-0.2gm	0.1-0.2gm	0.1-0.2gm	0.1-0.2gm
II)	**MA**	MA+SB+Ga	TJA+SB+Ga	WFA+SB+Ga	PAJA+SB+Ga
	TJA				
	WFA	100ml	-	-	-
	PAJA	-	100ml	-	-
	Sodium	-	-	100ml	-
	benzoate+Ganoderma	-	-	-	100ml
	extract (SB+Ga)	0.05+0.1gm	0.05+0.1gm	0.05+0.1gm	0.05+0.1gm
III)	**MA**	100ml	-	-	-
	TJA	-	100ml	-	-
	WFA	-	-	100ml	-
	PAJA	-	-	-	100ml
	Sodium benzoate (SB)	0.1-0.2gm	0.1-0.2gm	0.1-0.2gm	0.1-0.2gm

MA=Milk Agar TJA=Tomato Juice Agar WFA=Wheat Flour Agar
PAJA=Pine Apple Juice Agar SB=Sodium Benzoate Ga=*Ganoderma*

Result and Discussion

Growth of microorganisms on natural media supplemented with sesqiterpenoid extract of Ganoderma *Reissi*

All plates were observed after 10 days of incubation. The sesquiterpenoid extract of *Ganoderma* at 0.2% concentration significantly inhibited the growth of all microorganisms on natural media (P>0.01). These results were comparable with inhibitory effect of sodium benzoate at 0.1% and 0.2% concentration, *Ganoderma* extract 0.1% with sodium benzoate 0.05%. The sodium benzoate at 0.2 % concentration fully inhibited the growth of all microorganisms. Similarly combination of *Ganoderma* extract (0.1%) and sodium benzoate (0.05%)] completely inhibited the growth of all microorganisms in all media (P>0.01).

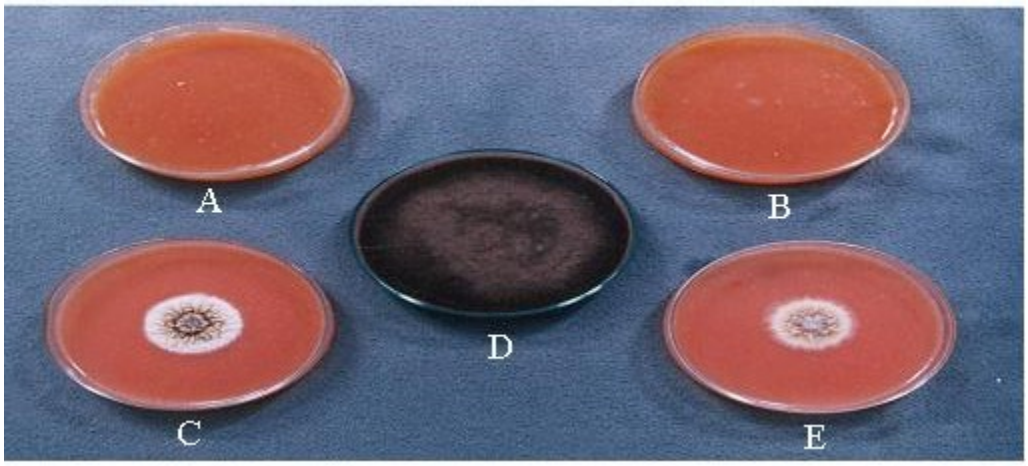

Fig.1 Inhibition of fungal growth on tomato agar supplemented with sesquiterpenoid extract of *Ganoderma* and sodium benzoate.
A:Medium supplemented with sesquiterpenoid extract of *Ganoderma* (0.2%), B: sesquiterpenoid extract of *Ganoderma* (0.1%) + sodium benzoate (0.05%), C: sesquiterpenoid extract of *Ganoderma* (0.1%), D: Without supplement, E: sodium benzoate (0.1%).

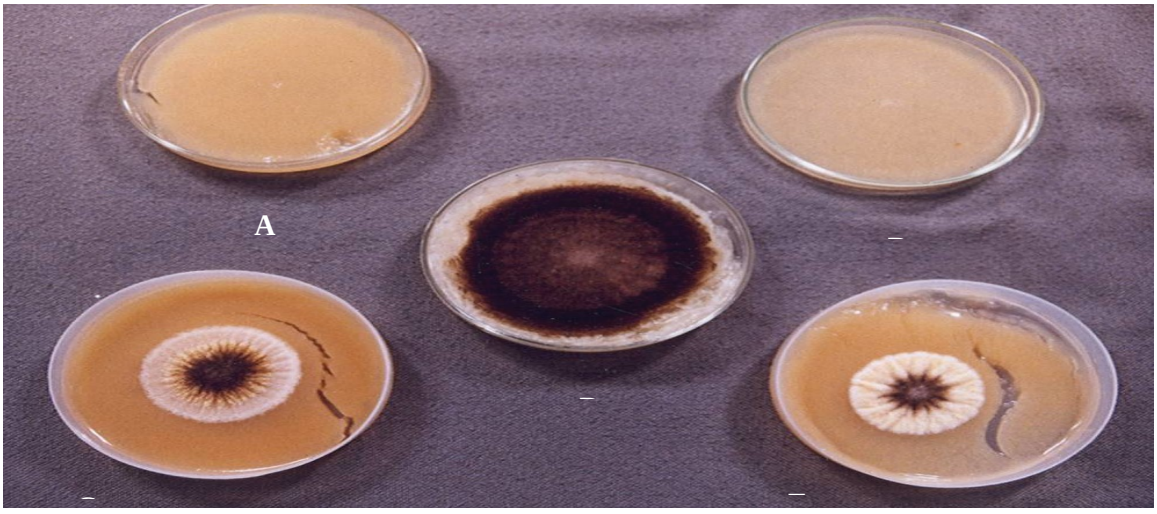

Fig.2 Inhibition of fungal growth on pine apple juice agar supplemented with sesqiterpenoid extract of *Ganoderma* and sodium benzoate.
A: Medium supplemented with sesquiterpenoid extract of *Ganoderma* (0.2%), B: sesquiterpenoid extract of *Ganoderma* (0.1%) + sodium benzoate (0.05%), C: sesquiterpenoid extract (0.1%), D: Without supplement, E: Sodium benzoate (0. 1%).

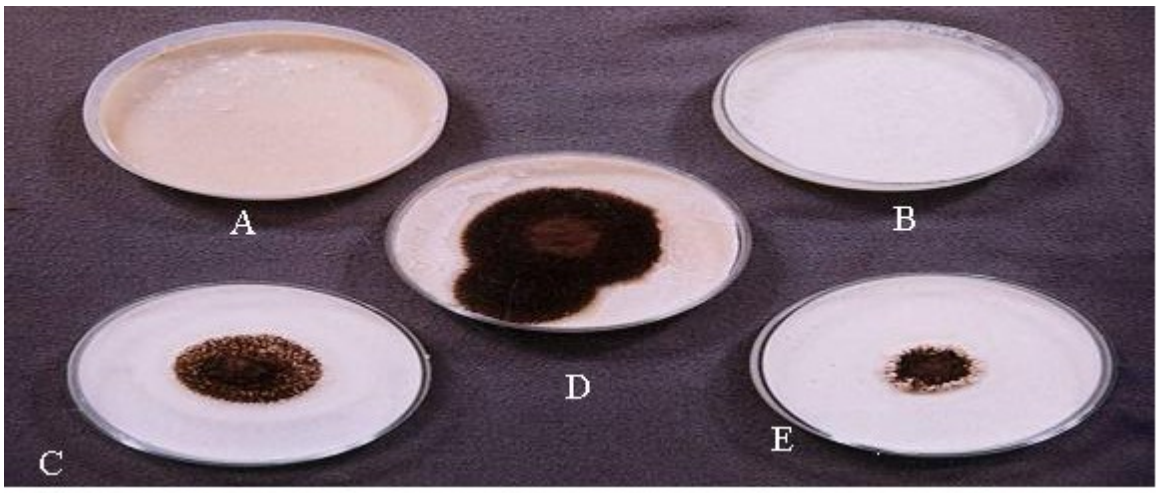

Fig.3 Inhibition of fungal growth on milk agar supplemented with sesqiterpenoid extract of *Ganoderma* and sodium benzoate.
A: Medium supplemented with sesqiterpenoid extract of *Ganoderma* (0.2%), B: sesqiterpenoid (0.1%) + sodium benzoate (0.05%), C: sesqiterpenoid extract (0.1%), D: Without supplement, E: sodium benzoate (0.1%)

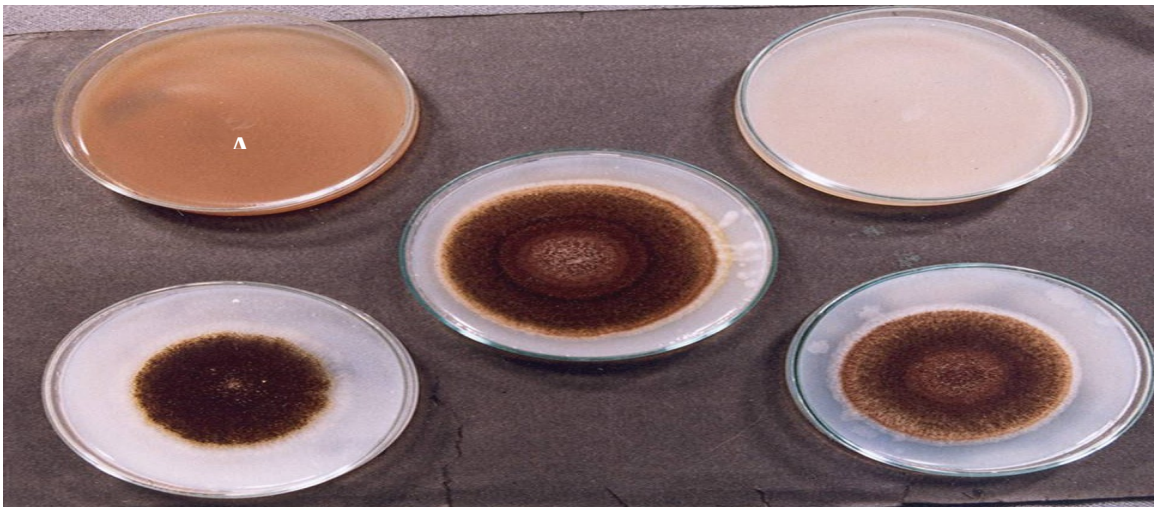

Fig.4 Inhibition of fungal growth on wheat flour agar supplemented with sesqiterpenoidextracte of *Ganoderma* and sodium benzoate**.**
A: Medium supplemented with sesquiterpenoid extract of *Ganoderma* (0.2%), B: sesquiterpenoid extract of *Ganoderma* (0.1%) + sodium benzoate (0.05%), C: sesquiterpenoid extract (0.1%), D: Without supplement, E: Sodium benzoate (0.1%).

Ganoderma is a basidiomycete white rot fungus which has been used for remedial purposes for centuries particularly in China, Japan and Korea (9). In the present study, the ability of sesquiterpenoid extracts of *Ganoderma Reissi* to inhibit food product spoilage by fungi was compared with chemical preservative (sodium benzoate) at same concentration (0.1%).

Increasing the concentration of sesquiterpenoid extract in the media to 0.2% completely inhibited the growth of fungi. The media used were similar to food products that are available in the market, like bakery products, dairy products, jams, and tomato sauce and fruit juices. Application of sesquiterpenoid extract of *Ganoderma* as a natural preservative, which has been extracted from *Ganoderma Reissi* may be a good alternative for chemical preservatives.

Recently, it has also been established that ochratoxin A is produced by fungal species, such as *A. niger* (10), which has also been found as a contaminant of bread. Different types of wheat and rye bread are made using sourdough (11). Sourdough is defined as dough with microorganisms, mainly lactic acid bacteria and yeasts, originating from sourdough or a sourdough starter and is metabolically active or need to be reactivated. Sodium benzoate is commonly used as a chemical preservative in different food items with maximum concentrations reported to be up to 2000 mg / kg of food materials (12). Findings of the present study showed enhancement of antimicrobial effect of sodium benzoate at 0.05% levels when used in combination with sesquiterpenoid extract of *Ganoderma* at 0.1% concentration. Findings of the current study show that combination of sesquiterpenoid extract of *Ganoderma* reduces the quantity of sodium benzoate to be used in food items for preservation. Therefore, practical application of *Ganoderma* extract as a preservative in bakery and other food products may be more effective than that of other food preservative.

Application of sesquiterpenoid extract of *Ganoderma* as a natural preservative may prove to be good alternative for food preservation. However more studies are needed to explore the details of the effective component of the extract and also to look at any side effects which may this extract have when consumed with foods.

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