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RESEARCH ARTICLE

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF SECONDARY METABOLITES OF *STEMPHYLIUM RADICINUM* (MEIER, DRECHSLER AND EDDY) ISOLATED FROM SOILS

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ABSTRACT

Background: A fungus isolated from soil displayed considerable antibacterial activity. The fungus was identified as *Stemphylium radicinum* (Meier, Drechsler and Eddy, 1922) based on morphological characterization.
Methods: Fungal extraction was carried out by ethyl acetate solvent. The metabolite showed Bioactivity against five bacterial strains. *Escherichia coli* from urine, *Staphylococcus aureus* from otitis media, *Streptococcus pyogenes* from Throat, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* from burns by using a disc diffusion technique was examined.
Results: The inhibition zones exhibited by fungal extract were 14.0 - 26.0 mm MIC test revealed that the extract of *S. radicinum* exhibited a minimal inhibition values ranging between (3.12 - 12.5 ug/ml) and MBC ranging between (6.25 - 50.0 ug/ml) against bacterial strains. A verification of non-toxicity of the fungal extract against human blood revealed a negative test. The chemical analysis of the fungal crude extract showed that extract *S. radicinum* contains Tannins group, phenol compounds and amino acid, absent flavenoide.
Conclusion: The metabolite produced by the fungi could be an alternative source of antimicrobial agents against clinical pathogens.

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INTRODUCTION

Secondary metabolites are small biomolecules considered to be non-essential for the life of the producer organism (Rodrigues, *et al*., 2000). Relatively few types of microorganisms produce the majority of secondary metabolites. Secondary metabolites are produced when the cell is not operating under optimum conditions for example when primary nutrient source is depleted. Secondary metabolites are synthesized for a finite period by cells that are no longer undergoing balanced growth (Kempken and Rohlfs, 2010). Most secondary metabolites are produced by some families as closely related compounds. The chemical structure and their activities cover a wide range of possibilities, including antibiotics, ergot alkaloids, quinolines, naphtalenes, phenazines, terpenoids, peptides and some complex growth factors.

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The production of economically important metabolites as antibiotics by fermentation of microbes is one of the major activities of the bioprocess industry. Secondary metabolites such as penicillin are produced during the stationary phase of cell growth. Most of the knowledge concerning secondary metabolism comes from the study of commercially important microorganisms (Calvo, *et al*., 2002; Brase, *et al*., 2009 and Barrios–Gonzaliez *et al*., 2003). Most fungal secondary metabolites are synthesized from only a few key precursors in pathways that comprise a relativelysmall number of reactions and which branch off from primarymetabolism at a limited number of points. Acetyl‑CoA is the most precursor of fungal secondary metabolites, leading toterpenes, polyketides, steroids and metabolites derived from fatty acids and Other secondary metabolites are derived from intermediates of the shikimic acid pathway, the tricarboxylic acid cycle and from amino acids (Dreyfuss and Chapela, 1994). Secondary metabolism has largely been the domain of organic chemists. Secondary metabolites are sometimes bioactive, usually of low molecular

weight, and are produced as families of related compounds at restricted parts of the life cycle, with production often correlated with a specific stage of morphological differentiation (Barrios–Gonzaliez *et al*., 2003). Secondary metabolites are well known for their ability to inhibitor other organisms. Terrestrial fungi served an enormous scope for the discovery of novel natural products in the past 60 years, many of them being potential targets for biomedical developments. The discovery of penicillin in 1929 started the era of fungal antibiotics and was followed by other important fungal metabolites for example cephalosporins and griseofulvins (Martin and Demain, 1980 and Kamba and Hassan, 2010). Several antibiotics have been discovered from the secondary metabolites produced by actinomycetes and fungi (Anke, 1989 and Pohanka, 2006). Nevertheless, during the last decade the research interests were focused on the activity compounds from fungi (Anke, 1989). This search for bioactive secondary metabolites has continued unabated, and thousands of compounds that inhibit the bacterial growth. Antioxidants act as radical-scavengers, and inhibit lipid peroxidation and other free radical-mediated processes; therefore, these are able to protect the human body from several diseases attributed to the reactions of radicals. Use of synthetic antioxidants to prevent free radical damage has been reported to involve toxic side effects thus necessitating the search for natural antioxidants and free radical scavengers (Radulovic *et al.,* 2007). Some synthetic antioxidant compounds like butylated hydroxytoluene, butylated hydroxyanisole and tertiary butylhydroquinone commonly used in processed foods. The natural antioxidants were characterized from the fungal compounds (Sun *et al*., 2004). Both natural and synthetic are gaining broad significance in prevention of diseases. To date, many kinds of bioactive compounds have been isolated from various fungi (Vatcharin *et al*., 2008).

MATERIALS AND METHODS

Fungal isolate

Soil samples were collected from different cultivated localities in Missan (southern Iraq) during the year 2014, brought to the laboratory and processed for fungal isolation. Soil dilution plating technique was applied for isolate of fungi from soil samples using Potato Dextrose Agar (PDA). Incubation was conducted at 27◦C for 5- 7 days. Fungal cultures were confirmed according to the available taxonomic literature (Domsch, 1980 and Ellis and Goodacre, 2012).

Extraction of secondary metabolites

Five discs (0.5 mm diameters) were cut from the axenic fungal culture of each isolate by using a cork borer were amended into PD liquid medium in 500 ml flasks (triplicates) and incubated at 27 ◦C for 14 days on a rotary shaker. Fungal cultures were filtered on what man No 1 filter paper and the pH was adjusted at 3 by HCl for fungal filtrate. Filtrate was extracted with ethyl acetate (1:1 v:v) by using separating funnel. The organic layer was collected by dehydration of water by using Na2 SO4. The filtrate was filtered again and placed in Petri dishes then left to be dried at room temperature. 100µg of the dried extract was dissolved in 1 ml ethanol as stock extract solution to be used for further experiments.

The test microorganisms

Esherichia coli from urine*, Staphylococcus aureus* from otitis media*, Streptococcus pyogenes* from Throat*, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* from burns were isolated from clinical cases in Sadder hospital in Missan (southern Iraq) and identified in their laboratories. The organisms were cultured on maintenance media until use.

Antimicrobial bioactivity assay

Filter paper discs (0.6 mm) after being sterilized by autoclave were socked in fungal crude extract solution for 5 min., filter paper discs with extract were placed on the surface of Muller-Hinton agar medium in Petri-dishes streaked with 0.2 ml of bacterial suspensions of Bacteria strains. Plates were incubated at 37 ◦C for 24 hr, an appearance of inhibition zones around the filter paper disc indicating the bioactivity of crude metabolites of the tested fungal isolates (Casals, 1979). The diameters of the clear zones were measured and compared with control agar plates containing discs with solvent only (control), triplicates were made.

Minimal inhibitory concentration and minimal bactericidal concentration test

The minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) values were determined by the standard serial dilution assay of the extract (McGinnis, 1980). Crude extract funguses S. radicinum isolate were selected for this test. The inhibitory test was carried out on Muller-Hinton agar medium.

Effect of storage period on the fungal extract bioactivity

Fungal extract of *S. radicinum* were kept in screwed vials at 4◦C for six months storage period to examine the bioactive longevity against both bacteria. Disc diffusion technique was applied and the inhibition zone diameters were measured.

Chemical analysis of fungal crude extract

Fungal culture extract of *S. radicinum* was chemically analyzed for alkaloids, phenols, amino acids, flavenoides and tannins according to following the method described by (Harborne, 1993).

Toxicity test

Cytotoxicity of the fungal crud extract was examined by using human RBC following a previously described method (Xian-guo and Ursula, 1994).

Antioxidant activity

The DPPH radical scavenging capacity was measured according to (Hatano *et al*., 1988). 1 ml of fungal extract was mixed with 0.5 ml of 0.2 mM methanolic DPPH solution. The reaction was allowed to stand at room temperature in the dark for 30 min and the absorbance was recorded at 517 nm against a blank (methanol solution). Dilutions were made to obtain concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, 7.81,

3.90 and 1.95 µg/ml. Tests were carried out in triplicate. The ability to scavenge the DPPH radical was calculated using the following equation:

Scavenging effect (%) = [(A0-A1)/A0] × 100

Where A0 and A1 are the absorbance of the control and the sample, respectively.

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA) between any pair of variables.

RESULTS

The strain was isolated from the soil of *S. radicinum.* Preliminary antibacterial screening was carried out on the crude extract of fungus *S. radicinum* against the six bacterial pathogens. The antibacterial effect of the fungus extract is shown in (Table 1), (Figs.1).

Table 1. Growth inhibition zones (mm diameter) exhibited by the fungal culture filtrate extract against five isolates of bacteria

Bacterial strains	Inhibition zones (mm)
Esherichia coli	14.0
Staphylococcus aureus	25.5
Streptococcus pyogenes	24.0
Klebsiella pneumoniae	26.0
Pseudomonas aeruginosa	0

Numbers represent average of three replicates P ≤ 0.05

Table 2. The minimal inhibitory concentrations (MIC) and minimal Bactericidal concentrations (MBC) of fungal crude extracts against isolates of bacteria

Bacterial strains	MIC(ug/ml)	MBC(ug/ ml)
Esherichia coli	6.25	25.0
Staphylococcus aureus	6.25	12.5
Streptococcus pyogenes	3.12	6.12
Klebsiella pneumoniae	3.12	6.12

Table 3. Bioactivity (inhibition zones mm diameter) of the fungal extract stored at 8 ◦C for six months against isolates of bacteria

Bacterial strains	Inhibition zones (mm)
Esherichia coli	10.0
Staphylococcus aureus	22.0
Streptococcus pyogenes	20.0
Klebsiella pneumoniae	21.0

Numbers represent average of three replicates ≤ 0.05

Table 4. Chemical compound groups of the fungal crude extract

Fungal species	Alkaloides	Amino acids	Flavenoides	Phenols	Tanins
S. radicinum	+	+	-	+	+

+ Present – absent

DISCUSSION

The inhibition zone values were highly variable, ranging between 14.0-26.0 mm except *Ps. aeruginosa* was resistant.

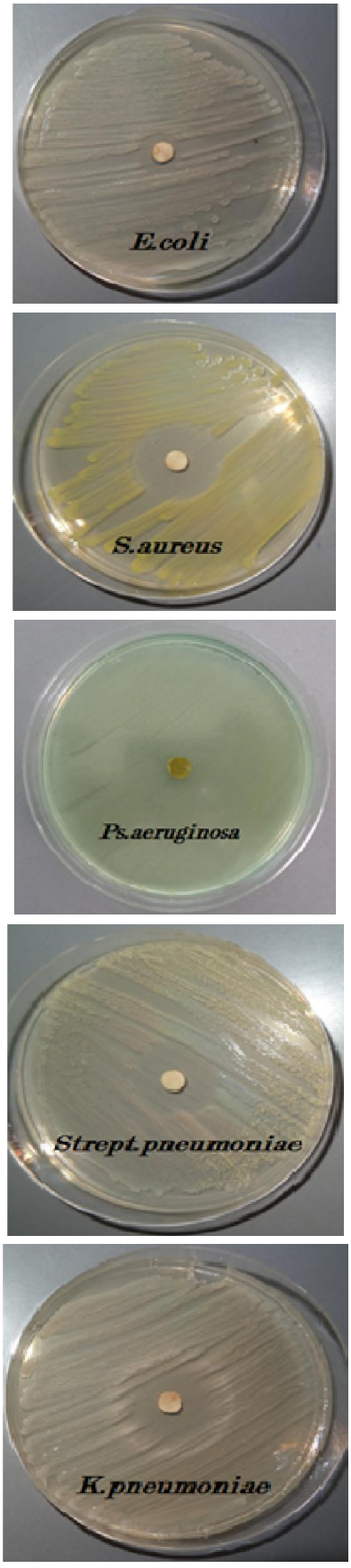

Fig.1. Inhibition zones exhibited by fungal crude extract against bacterial strains

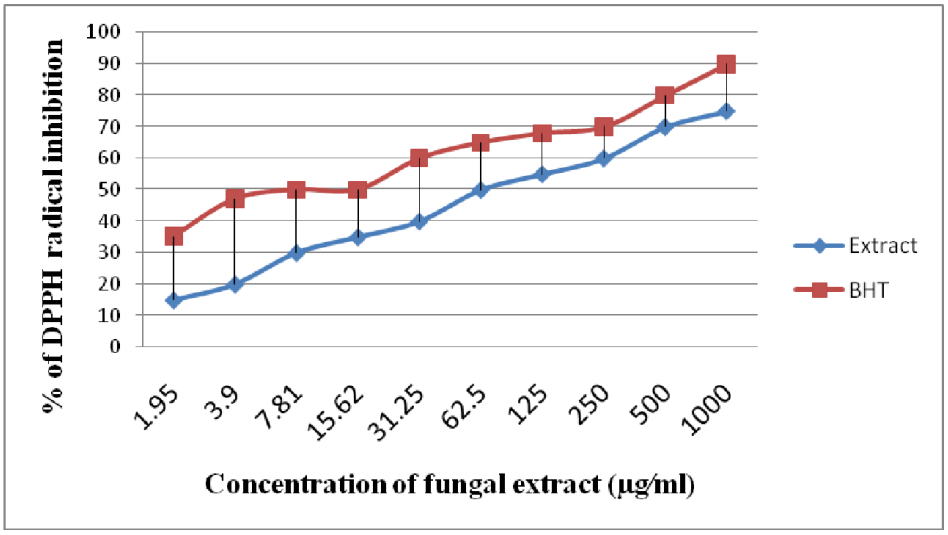

Figure 2. Antioxidant (DPPH scavenging) activity of investigated fungal extract presented as percentage of DPPH radicals inhibition

The fungi in general are a good source for antimicrobial agents (Janes *et al*., 2007). It has been investigated that some species of this genus are sources for a potential secondary metabolites such as phytotoxins, Stemphyloxin I, $C_{12}H_{32}O_5$. Nevertheless, the production of secondary metabolites substances by fungi, in general, is often affected by various growth conditional factors mainly the fermentation medium (Vahidi *et al*., 2004). In the present study a liquid state fermentation medium used is efficient for a mass production of bioactive secondary metabolites by the fungus. The crude filtrate extract of the examined of *S. radicinum* exhibited an inhibitory action against a gram positive and gram negative bacteria. A conclusion can be derived from this preliminary screening of *S. radicinum* that this fungus possessing a potential secondary chemical compounds that can be of significance and a promising as antimicrobial agents. However, a further investigation is needed to test the bioactivity of these extract against more pathogenic bacteria and pathogenic fungi as well. The MIC and MBC tests indicated that the extract of *S. radicinum* exhibited the minimal values of MIC ranging between (3.12 - 12.5 ug/ml) and MBC ranging between (6.25 - 50.0 ug/ml) against bacterial strains. A verification of non-toxicity of the fungal extract against human blood revealed a negative test. The bioactivity of the fungal extract kept at 8 ◦C for six months storage period showed insignificant difference compared to the initial (fresh) fungal extract. Nonetheless, slightly lower values of growth inhibition zones were noticed for the fungal extract after being stored for six months. However, amongst the screened fungal extract exhibited highest inhibition zones diameters before and after the storage period. The chemical analysis of the fungal crude extract showed that extract *S. radicinum* contains Tannins group, phenol compounds and amino acid, absent flavenoide. The antimicrobial inhibitory impact of these extracts can be related to the bioactivity of these compounds. It has been reported that several phenolic compounds including tannin are potent inhibitors of microbial enzymes (Kamba and Hassan, 2010). Studies showed that tannin inhibits the growth of bacteria and has been attributed to the mechanism of tannin binding with the protein of the bacterial cell walls (Shihabudeen *et al*., 2010).

On the other hand, the inhibitory action of alkaloides against both G-negative and G-positive bacteria has also been demonstrated. Nonetheless, the inhibitory mechanism has been related to the inhibition of DNA synthesis by specific alkaloid compounds (Sawer *et al*., 2005). Evidently, the effect of storage for six months did not significantly alter the antibacterial activity of these extract against the tested bacteria. Antioxidants are compounds that inhibit or delay the oxidation process by preventing the initiation or propagation of oxidizing chain reactions. DPPH radical scavenging assay is a swift and sensitive method for the antioxidant activity. The determination of free radical scavenging activity using the stable 2, 2-diphenyl-1-picryl-hydrazyl radical (DPPH) has received the utmost attention owing to the ease of use and its convenience (Agostini-Costa *et al*., 2012). It was observed that the scavenging activity of secondary metabolites from *S. radicinum* at all concentrations from1.95 to 1000 µg/ml is rather strong (15-75%). The extract improved 75% inhibition at higher concentrations, indicating lesser antioxidant capacity than positive control.

Conclusion

Potential of soil fungi have received tremendous attention due to the fact that, Isolation of soil fungi might present bioactive compounds bearing pharmaceutical importance. In order to harness these plethora of microorganism much studies are to be awaited across the globe which could exert huge impact on life expectancy. Hence in the present investigation preliminary work has been carried out towards isolating soil fungi plethora for further antimicrobial assay. We identified fungus *Stemphylium radicinum* antibacterial property which act against medical important pathogen. Bioactive compounds were produced bysoil fungus *Stemphylium radicinum* which were extracted with ethyl acetate, showed potent scavenging activity on DPPH free radical comparable with the standard antioxidant. Antioxidant activities of the extract from fungus *Stemphylium radicinum* is mainly attributed to the active compounds present in them. The results obtained showed that the fungus *Stemphylium radicinum* extract can be considered good sources of natural antioxidants.

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