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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF ALKALOID EXTRACT FROM THE LEAVES OF *ZYGOPHYLLUM FABAGO*.

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Abstract

Due to the risk properties of commercial biocides, plant-based biocides may propose a safer alternative for control microbes that cause sick building syndrome and building deterioration. The aim of the study was to determine a new source of bioactive extracts from plants. Alkaloid extract from leaves of *Zygophyllum fabago*, was screened for potential antimicrobial activity against one species of bacteria (*Bacillus subtilis*) and four filamentous fungi (*Aspergillus flavus*, *Aspergillus niger*, *Penicillium oxalicum* and *Cladosporium oxysporum*) isolated from enclosed sick buildings. The minimum inhibitory concentration against the highly sensitive microorganisms i.e. *B. subtilis* and *P. oxalicum* was 0.157 and 0.635 mg/ml, respectively.

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Introduction:-

Microbes, mostly fungi and bacteria; on building materials and indoor surfaces represents indoor air quality problems (Chester and Levine, 1997; Zhu *et al.*, 2004). Species of fungi and bacteria have been isolated from indoor damaged building materials (Hyvärinen *et al.*, 2002). These organisms were caused odour, defacement and deterioration to the building materials (Srikanth *et al.*, 2008). Many of these microorganisms were related to allergic and irritant reactions, infectious disease respiratory problems, hypersensitivity reactions, cancer and leukemia on the building residents (Green and Scarpino, 2002); (Burge, 2004).

Controlling of these microorganisms using active materials such as synthetic biocides has been limited due to their harmful effects on the environments, deposit problems and their carcinogenicity (Verma *et al.*, 2008). Thus, the search for ways to control and treatment the effects of microbes in an eco-friendly way is a need. Therefore, biocides derived from plants represents a safer, more active and environment-friendly alternatives for microbial control because they are rich in bioactive phytochemicals (Cooley *et al.*, 1998).

Zygophyllum fabago belongs to the family Zygophyllaceae, order Geraniales (Bhattacharyya and Johri, 1998), commonly called Bean-Coped or Syrian bean-caper (Jose-Abod *et al.*, 2007), is a sub-shrub distributed in tropical and sub-tropical region in south Europe, North Africa and West-South Asia including Iraq (Al-Mokhtar, 1971). *Z. fabago* extract is used as antitussive, expectorant, anti-inflammatory and for removing pains (Feng *et al.*, 2008). It is also used as a part of drug for rheumatism and goat; also used extremely as poultice to cure skin diseases, external wound and injuries (Khan *et al.*, 2014). Many researches showed about antimicrobial activity of *Z. fabago* against bacteria and fungus but by using crude extract (Zaidi and Crow, 2005; (Dana *et al.*, 2010; Mohammed *et al.*, 2013;

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Abdel-Hamid, 2014).). In this study we test a new source of plant-based preparations, available in our environment and examine fractionation compounds (Alkaloids) for remediation of microbial aerosols.

Materials and Methods:-

Plant Material:-

Zygophyllum fabago leaves were collected in Baghdad in June 2016. The botanical identification was made by comparing with authentic herbarium specimen at the herbarium of Botanical Garden in Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad.

Source of Microorganisms:-

Four species of filamentous fungi and one species of Gram-positive bacteria isolated during a series of indoor samplings in air-conditioned sick buildings Wardah *et al.* (2011) were obtained from the culture collection unit of the Plant Pathology Laboratory, School of Biological Sciences, Universiti Sains Malaysia. The microorganisms were identified as *Aspergillus flavus* (AP22BA), *A. niger* (AP29BA), *Penicillium oxalicum* (PP1BA), *Cladosporium oxysporum* (CP1BA) and *Bacillus subtilis* (BP1BA) and kept in 15% glycerol at -86 °C (Salleh and Sulaiman, 1984).). The work cultures of bacterium and fungi were maintained at 4 °C on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) slants, respectively.

Extraction and Isolation:-

The crude alkaloidal extracts from the plants were obtained according to the method of Harborne (1984) and (Zaree *et al.*, 2013) with slight modifications. Dried and powdered leaves of *Z. fabago* (1.5 kg) were extracted with 80% ethanol in a Soxhlet apparatus and concentrated to a dark brown gummy residue (349.5 g). The extract was then treated with 5% aqueous HCL to pH and extracted with CHCl₃ (3 X 800 ml). Next, the aqueous phase was basified to pH -10 with 25% NH₄OH and extracted repeatedly with CHCl₃ (3 X 800 ml). The alkaline solution remaining after removal of CHCl₃ fraction was treated with an excess of Mayer's reagent until precipitation ceased. The resulting precipitate was filtered by suction, washed with water, dissolved in 100 ml methanol and passed through a column of anion exchange resin (Cl) which was preconditioned before use with 5% HCl. The column was then rinsed with 500 ml methanol and the column eluent and rinsing were combined to afford a residue of crude quaternary alkaloid chloride fraction (1.529 g). Thin-layer chromatography of this fraction revealed the presence of one spot (Rf 0.54) (Henrickson *et al.*, 1997).

Antimicrobial activity test:-

The antimicrobial activity was carried out by disc diffusion method Murray *et al.* (1995) with a slight modification in volume and concentration of tested compound. Briefly, 0.1 ml of suspension containing of 10⁸ CFU/ml of bacterium and 10⁴ spore/ml of fungi spread on Nutrient Agar and potato dextrose agar (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 liter), respectively. Sterilized Whatman AA discs containing 20 µl of tested compound (100 mg/ml in DMSO) were placed into the inoculated agar. The quantity of compound was 2 mg/disc. Equal volume of DMSO was assayed as negative control. The sample was tested against each organism in triplicate. The plates were incubated at room temperature (28 ± 2°C) for 28 h in case of bacterium and 3-5 days for fungi. The diameters of inhibition zones around discs were measured using ruler and expressed in millimeter (mm) unit.

Determination of Minimum Inhibitory Concentration (MIC):-

The minimum inhibitory concentration of the active compound against the highly sensitive microorganisms i.e. *B. subtilis* and *P. oxalicum* were determined by agar dilution method (Mukherjee, 2002).) with a slight modification. In treated plates, the amount of the compound was dissolved in 1 ml sterile distilled water and then mixed thoroughly with 9 ml of molten agar at 50 °C in pre-labeled sterile Petri dishes to obtain a series of final concentrations of compound between 0.625- 0.0097 mg/ml for *B. subtilis* and 5-0.312 mg/ml for *P. oxalicum*. A control with sterile distilled water was also set up. About 20 µl of suspension containing previously 10⁸ CFU/ml of bacterium and 10⁴ spore/ml of fungi was used to inoculate the plates. The plates were then incubated at room temperature (28 ± 2°C) for 48 h in case of *B. subtilis* and 72 h for *P. oxalicum*. The lowest concentration of the compound at which there was no visible growth of a microorganism after incubation was taken as MIC (Banso and Adeyemo, 2006).).

Statistical Analysis:-

Data analyzed with SPSS software to know the difference among the microorganisms. All determinations were made in triplicates and the data is reported as mean ± SE for (n = 3). Analysis of Variance (ANOVA) method was used for statistical analysis and at probability levels (0.05, 0.01, 0.001) for the purpose of evaluating the differences

in the results of transactions in terms of being significant (influence of material) or not significant differences (as a result of laboratory errors) (SAS., 2012).

Results and Discussion:-

Z. fabago leaves extract, showed a positive test results (orange sediment) with Dragendorff's reagent, this finding agree with Mohammed *et al.* (2013); Abdel-Hamid (2014); Sabahi *et al.* (1985) and Torssel (1997) which mentioned presented alkaloid compound in *Z. fabago* leaves. Abdel-Hamid (2014) determined the amount of alkaloids in leaves of *Z. fabago* in about 0.44% from the total contents while in this study the amount of alkaloids extract was approximately 0.102%, this different in amount may due to the variation of area of isolation, season and time of collecting (Jayanthi *et al.*, 2013).

The alkaloid extract was tested against one species of bacteria (*B. subtilis*) and four filamentous fungi (*A. flavus*, *A. niger*, *P. oxalicum* and *C. oxysporum*) that were isolated from sick enclosed buildings and showed a significant effectiveness in which the level of probability was less than (0.001, 0.05, 0.01). Using the simple disc diffusion assay, this compound exhibited zones of inhibition of at least 14.35 ± 0.43 mm diameter against all the microorganisms at a loading of 2 mg/disc (Table 1).

Table I:- The diameter of inhibition zones of the isolated alkaloid.

	Diameter of inhibition zone (mm)*				<i>P. oxalicum</i>
	<i>B. subtilis</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>C. oxysporum</i>	
Isolated alkaloid	24.69±0.50	14.35±0.43	15.34±0.05	15.66±0.21	16.68±0.33

* refers to inhibition zone including the diameter of disk paper (6 mm).

Values are mean inhibition (mm) ± S.E. of three replicate.

The minimum inhibitory concentration (MIC) values of this compound were also determined against the highly sensitive microorganisms i.e. *B. subtilis* and *P. oxalicum* using agar dilution method. The results (Table 2) showed that this compound was the most potent against *B. subtilis* with MIC of 0.157 mg/ml.

Table II:- MIC values mg/ ml of the isolated alkaloid

	MIC (mg/ ml)	
	<i>Bacillus subtilis</i>	<i>Penicillium oxalicum</i>
Isolated alkaloid	0.157	0.635

The results showed that the treatment of fungi, *Aspergillus niger*, *Aspergillus flavus*, *Caldosporium oxysporum* and *Penicillium oxalicum* and bacteria *Bacillus subtilis* with *Z. fabago* alkaloids extract have antimicrobial activity in which the level of probability was less than (0.001, 0.05, 0.01). This results agreed with Zaidi and Crow (2005) which found that the methanolic extracts of *Z. fabago* have a highly effect against *Candida albicans* (Fungus) and *Escherichia coli* (Bacteria), and agreed with Dana *et al.* (2010) who mentioned that there was an inhibitory effect of aqueous extracts of *Z. fabago* leaves on two plant phytopathogenic fungal species (*Fusarium oxysporum* f. sp. *melonis* and *Pythium aphanidermatum*) which were studied under laboratory conditions, the maximum mean inhibition was recorded with the 10% w/v extracts was 42.9% and 85.3%, respectively. Moreover, the cytotoxicity of *Z. fabago* leaves extract was observed against *C. albicans* at different concentrations of extracts for a period of 1, 2, and 24 h at 25°C, the 1 h exposure showed that 1.0 g/ml extract of *Z. fabago* killed 98% of cells (Zaidi and Crow, 2012). Study Mohammed *et al.* (2013) found that the aqueous and alcoholic extracts of *Z. fabago* leaves have antimicrobial activity against eight species of pathogenic bacteria (*Escherichia coli*, *Klebsilla Pneumonia*, *Proteus vulgaris*, *Proteus mirabilis*, *Salmonella* sp., *Pseudomonas aeruginosa*, *Streptococcus Pyogens*, *Staphylococcus aureus*) with six different concentration began with 40 mg/ml to 100 mg/ml by using agar well diffusion method which showed that alcoholic extracts was effective than aqueous ones and that both *Staphylococcus aureus* and *Escherichia coli* were the most affected species.

Results showed that the effectiveness of extracts were varied according to microorganisms, extracts were more effective against bacteria *B. subtilis*. This founding agree with Gnan and Sheriha (1986) which showed that the sensitivity of bacteria *B. subtilis* and *S. aureus* to alkaloids was more than other organisms. This inhibitory action can be explained by interaction of alkaloids with metabolic reactions necessary to growth and reproduction of

microorganisms by inhibition protein synthesis at levels of ribosome and distraction component of plasma membrane (lipid and protein) (Okoye, 2015).

Z. fabago contains two type from alkaloids (indole derivatives and anthranilic acid derivatives), the first type effect on amino acid glycine whereas the second type effect on RNA and DNA synthesis and inhibits of protein synthesis in addition to inhibition of ionic carriers and canal (Wink, 1997). In fungus, alkaloids inhibit chitin synthesis and sterol that are important in synthesis of fungus cell wall by inhibition of important synthesis wall enzyme like Sterol Δ^{23} -methyltransferase and Chitin synthase (Kang *et al.*, 1999).

Conclusion:-

In summary, the current study together with previous results are a significant step in developing plant-based microbial biocides which are eco-friendly in treatment airborne aerosols and consequently to be established commercially.

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