EFFECT OF SOME EXTRACTS OF *Funaria sp*. ON SOME TYPES OF BACTERIA

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ABSTRACT

The aim of this study is to investigate the effect of cold and hot water extracts of green parts of *Funaria sp.* on colonies growth of *Escherichia coli* and *Klebsiella sp.* pathogenic strain and find the half lethal concentration (LC_{50}). Also, it has been done in period from 1/8/2017 to 1/2/2018 under microbiological laboratory conditions. The results showed that cold water extract was more effective on bacteria than hot water extracts, which the LC_{50} in cold and hot water (2) mg/l and (< 0.5) mg/l, and (> 2.5) mg/l and (1.7) mg/l for *E. coli* and *Klebsiella sp.* respectively. *Klebsiella sp.* was more effective by extracts than *E. coli* bacteria in different concentrations. Results also showed there was a significant differentiation in level of probability 0.05 and negative correlation coefficient between results.

Keywords: MOSSES, EXTRACTS, COLONIES, LC_{50}

تاثير بعض مستخلصات *Funaria sp.* على بعض انواع البكتريا

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الخلاصة

هدفت هذه الدراسة الى التحقق من تاثير المستخلصات المائية الباردة والحارة للاجزاء الخضراء لجنس *Funaria sp.* على نمو مستعمرات السلالة الممرضة من بكتريا *E. coli* و *Klebsiella sp.* وايجاد قيمة التركيز القاتل لنصف احياء الاختبار (LC_{50}). كذلك اجريت الدراسة في الفترة من 1 / 8 / 2018 الى 1 / 2 / 2018 تحت ظروف مختبر الاحياء المجهرية .اذ اظهرت النتائج ان المستخلصات المائية الباردة كانت الاكثر تاثيرا على البكتريا من المستخلصات المائية الحارة والباردة, اذ كانت قيمة LC_{50} (2) ملغم/لتر و (< 0.5) ملغم/لتر و (<2.5) ملغم/لتر و (1.7) ملغم/لتر لمستخلصات الماء الحار لكل من بكتريا *E. coli* و *Klebsiella sp.* على التوالي. وان بكتريا *Klebsiella sp.* كانت الاكثر تاثرا بالمستخلصات من بكتريا *E. coli* عند تراكيز مختلفة. كما اظهرت النتائج بان هنالك فروق معنوية عند مستوى احتمالية 0.05 ومعامل ارتباط سلبي بين النتائج.

الكلمات المفتاحية: حزازيات , مستخلصات , مستعمرات , LC_{50}.

INTRODUCTION

Many studies around the world have been used materials from many genus of plants or few times animals to determine their effectivity against many types of disease factors that caused by microorganisms (1), and used it if possible instead of synthetics drugs that caused huge problems clinically and environmentally, which some study such as study of (2) found that aqueous and methanolic extracts of *Schizandrae fructus* leaves have been used in korea to treat gastrointestinal diseases by *Salmonella sp.* instead of manufactured drugs. For that a few studies suggested to wide range of searching among life forms to find suitable compounds that could be helpful and less dangerous on non-target organisms.

Funaria is genus belong to Kingdom of Plantae and Order of Funariales, is a common moss has located between vascular plant and algae. which has 117 species that were global and widely distribution in nature specially in tropical and temperate regions (3). It is known as cord, rope or green moss, which growth on moist ground, rocks, tree stems, and under shade as silky or velvety tuft. It has two types of life cycle which are sexual and asexual or vegetative type (4). Also, as a member of bryophytes it has been used to treat cuts, burns, wounds, neurasthenia, pneumonia and others. It could be used for their antibiotic activity against fungi and prokaryotic cells (5),

as well as the study of (6) appeared that extracts of 23 species of bryophytes have ability to inhibition of *Paenibacillus larvae* bacterial growth that cause disease in honeybee larvae.

Escherichia coli is gram-negative bacilli bacteria, a facultative anaerobe, non-spore, has or hasn't flagellate and can fermented simple sugars (7). Found naturally in soil or sometimes in digestive system of humans and animals. It has strains can cause disease such as enterotoxigenic strains while others could be deadly (8). Klebsiella sp. is Gram-negative bacilli bacteria, can be found naturally everywhere in environment, nonmotile, nonsporing, sometimes encapsulated, facultative anaerobic and positive in the Voges-Proskauer test. These genera consist of (77 K) antigens and (9 O) antigens that cause different serogroups (9). Also, they cause hospital infections and occur in mucoid colonies. Both bacteria belong to the large *Enterobacteriaceae* family (10).

This study aimed to know the effect of cold and hot water extracts of green parts of *Funaria sp.* mosses on colonies growth of *E. coli* strain pathogen and *Klebsiella sp,.* and find the LC_{50} value to improve the extracts effectivity after treatment the culture media treated by different concentrations of extracts.

MATERIALS AND METHODS

PREPARATION OF EXTRACTS

Similar method of (11) and the way followed by (12) have been used. The green parts of mosses that growth above ground have been collected and washed with fresh tap water to remove dust, dried for 24 hours at room temperature then grinded by electrical mill and sieved through clean passage gauze to remove the solid parts of dried parts that didn't grind well. To prepare cold extracts, weight of 10 gm of the preparing powder has been mixed well with 50 ml cold distilled water and for preparing of hot extracts, also 10 gm from powder has been mixed well with 50 ml hot distilled water and left for 10 minutes to boil. Each sample blended by an electric mixer. Both extracts separated first by normal filtaration papers and second by the centrifuge on speed of 3000 r / min for 15 minutes to have the filtrate; which was left to dry in room temperature to get dry green powder for each extract. Then 0.1 gm from dry green powder for each extract has been mixed separate with 500 ml of distilled water to obtain the stock solution that have 0.02 % concentration respectively to prepare (0.5, 1.5, 2.5) mg/l concentrations to each extract. At last, both aqueous extracts sterilized through millipore paper have 0.45 µm diameter holes and transferred to sterile bottles under refrigeration until the test.

PREPARATION OF BACTERIA

bacteria have been isolated from soil and diagnosis of pathogenic strain of *E. coli* and *Klebsiella sp.* by using a biochemical test (IMVIC test) and used Eosin Methylene Blue Agar (EMB) to growth and isolate pathogenic strain of *E. coli* in 55 C^o for 24-48 hours and MacConkey's agar to growth and isolate *Klebsiella sp.* in 37 C^o for 24-48 hours (13; 14). After that, both bacteria culturing and let to growth separately on MacConkey broth for 24-48 hours in 55 C^o and 37 C^o respectively then storage as stock in refrigerator to prepare for the test. Before beginning the test, the serial dilution to 10^3 have been made on each bacterium before mixing with agar culture that have aqueous extracts.

PREPARATION OF THE MEDIA

Depended on the similar method of (15), the MacConkey agar was used as culture media to both bacteria, the stock solution (0.02 %) have been used to prepare (0.5, 1.5, 2.5) mg/l to each extract. Also, the volume of distil water that used to prepare culture media have been calculated in concentration, then different concentrations of each extracts mixed separately with culture media. The control sample has been made by using culture media without any addition

THE CALCULATION OF LC_{50}

To study the effect of cold and hot water extracts of green parts of *Funaria sp.* on pathogenic strain of *E. coli* and *Klebsiella sp.* bacteria, the LC_{50} have been found after using series of concentrations (0.5, 1.5, 2.5) mg/l, in addition to control sample. The technique of pour plate count (14) has been used as a method to calculate average numbers of colonies that growth on culture media (MacConkey agar), in addition to control sample. The average number of colonies have been calculated after 24-48 hour at 55 C^o and 37 C^o to both bacteria respectively (16). Also, the lethal concentration for median or (half) lethal concentration (LC_{50}) has been calculated by using the equation of straight line [Y= bx + a (a= intercept, b= slope)] (17) after the data corrected with Abbott equation (18).

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

A completely randomized design (CRD) was used. Data were analyzed statistically by using less significant differences (LSD) at 0.05 after subjection to the analysis of variance. Also, the correlation coefficients have been found (19).

RESULTS AND DISCUSSION

The result appeared in Table (1) and Figure (1) that cold water extracts of *Funaria sp*. of new bioactive compounds such as isoflavonoids, were more effective on colonies growth of pathogenic strain of *E. coli* and *Klebsiella sp.* bacteria than hot water extracts in (0.5, 1.5, 2.5) mg/l concentration, which the percentage growth of colonies was (73, 57, 43) % respectively for *E. coli* and (28, 14, 7) % respectively for *Klebsiella sp.* in average growth of colonies (85, 66, 50) colony respectively for *E. coli* and (21, 11, 5) colony respectively for *Klebsiella sp.* bacteria. While the percentage growth of colonies on culture media that treated with hot water extracts (90, 75, 58) % respectively for *E. coli* and (67, 53, 36) % respectively for *Klebsiella sp.* in average growth of colonies (104, 87, 67) colony respectively for *E. coli* and (51, 40, 27) colony respectively for *Klebsiella sp.*. The results also showed that there was a significant differentiation and the average growth of colonies on cold and hot water extracts decreased with increasing concentrations with negative correlation coefficient.

The results in Figure (2) appeared low percentage growth of colonies on cold water extract to both bacteria with LC_{50} value was (2) mg/l and (< 0.5) mg/l for *E. coli* and *Klebsiella sp*. respectively. These results could be due to content cold water extracts on materials that have activity against bacteria, which some studies showed that mosses could be potential sources

Table (1) Average growth of colonies and percentage growth and mortalities to pathogenic strain of *E. coli* and *Klebsiella sp.* colonies after 24 hours of exposure to different concentration of cold and hot water extract of *Funaria sp.*

bacteria	Concentration Mg/l	cold water extract			hot water extract		
		Average growth of colonies[1]	Percentage of growth (%)	Percentage of mortalities (%)	Average growth of colonies[2]	Percentage of growth (%)	Percentage of mortalities (%)
*E. coli**	**Control**	116	100	-	116	100	-
	0.5	85	73	27	104	90	10
	1.5	66	57	43	87	75	25
	2.5	50	43	57	67	58	42
*Klebsiella sp.***	**Concentration Mg/l**	**cold water extract**			**hot water extract**		
		Average growth of colonies[3]	**Percentage of growth (%)**	**Percentage of mortalities (%)**	**Average growth of colonies[4]**	**Percentage of growth (%)**	**Percentage of mortalities (%)**
	Control	76	-	-	76	-	-
	0.5	21	28	72	51	67	33
	1.5	11	14	86	40	53	47
	2.5	5	7	93	27	36	64
LSD*=1.028 ; LSD**=0.608 ; r^1= -0.99 ; r^2= -0.99 ; r^3= -0.98 ; r^4= -0.99							

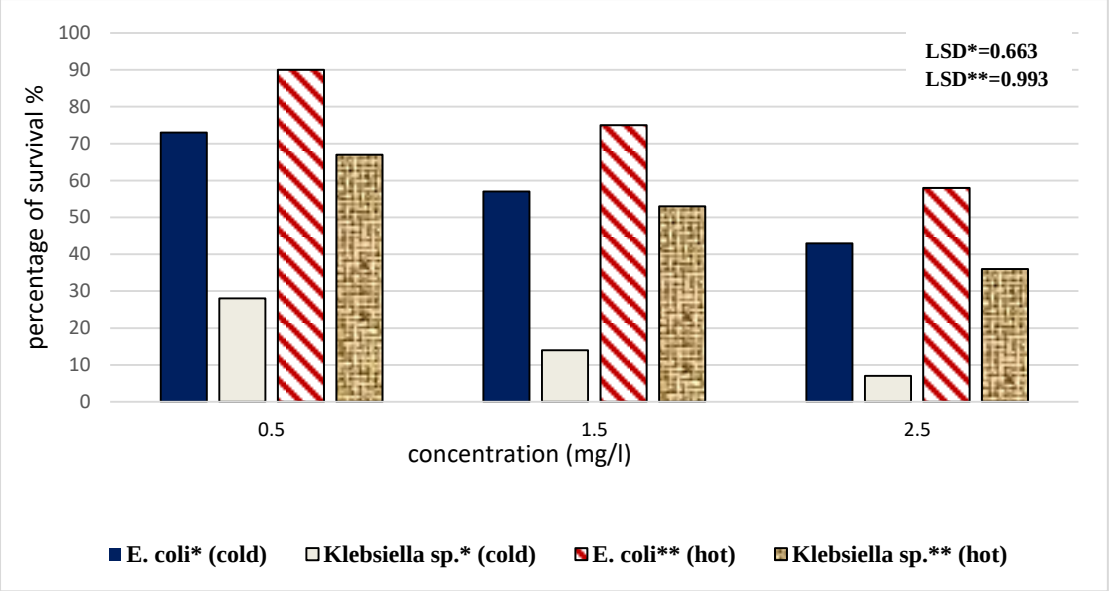

Figure (1) percentage growth of pathogenic strain of *E. coli* and *Klebsiella sp.* colonies on culture media treated for 24 hours with cold and hot water extracts of *Funaria sp.*

flavonoids, bioflavonoids, terpenoids, phenolic, volatile constituents, alkaloids, saponins and steroids worked as antibacterial to some sort of bacteria, and some of these materials have ability to destroy cell wall bacteria (20; 21), or bacterial growth inhibited by interfere with bioenzymatic system (22), or interfere with bacterial bioactivity (23). Other studies referred to these materials as a mechanism to prevent competition relationships (24), while others referred to it as a cooperative mechanism that allow to some type of bacteria to growth as exchangeable provide mosses some compounds or nutrient metals (25).

Results in Figure (2) also showed that *Klebsiella sp*. was more effective by compounds in cold water extracts than *E. coli* bacteria which the LC_{50} was less than 0.5 mg/l (the lower concentration that used), and the percentage growth of colonies appeared negative correlation coefficient (r=-0.98) with concentration. Many studies referred to these situation as a reason of penetration different materials through bacterial cell wall that increased with increasing concentration (26) and other studies referred to it as a cause of destroying cell wall structure (27) that makes many compounds easy to inter. Also, results noted to the presence of growth to *E. coli* and the existence of negative correlation coefficient (r=-0.99) with different concentration. This may be due to some strains of *E. coli* bacteria have wide genetic variety and massive genetic stocks (28) that make the bacteria more adaptive toward dangerous materials by lock it out, extraction or analysis by enzymes or by metabolism (29). In that state, the damage was less in case of penetrating materials inside the cell wall bacteria. While *Klebsiella sp*. bacteria as mention in some genetic studies have less variety in genetic content (30) than *E. coli*. However, some strains of *Klebsiella sp.* showed resistance that made some studies connected it with the ability to make diseases (31). Also, *Klebsiella sp*. showed average growth of colonies in different concentrations, where some studies pointed to posse some genes that gave some strain ability to survive in extraordinary conditions (32). These genes described sometimes as lethal pathogenetic genes (33), which formed most of genetic content of the bacteria and large part of bacterial plasmid or chromosome (34)

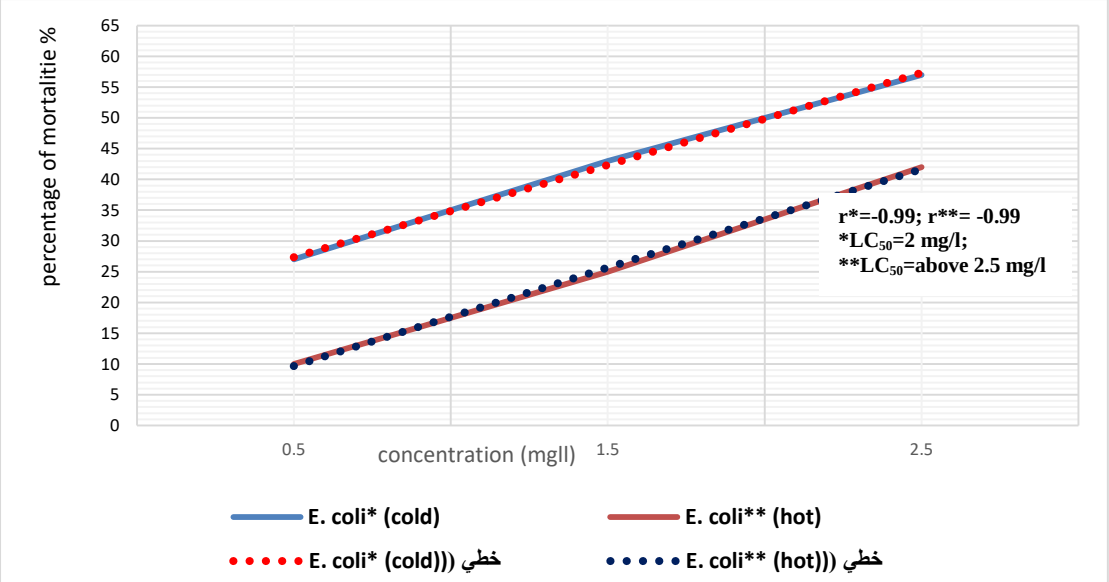

Figure (2) value of LC_{50} after 24 hours of treatment pathogenic strain of *E. coli* bacteria with cold and hot water extracts of *Funaria sp.*

The results appeared in Table (1) that *E. coli* and *Klebsiella sp.* have higher percentage and average growth of colonies on hot water extracts of *Funaria sp*. than cold water extracts, with LC_{50} value (> 2.5) mg/l and (1.7) mg/l for *E. coli* and *Klebsiella sp*. respectively. Which could be returned to the effect of temperature on some components of hot water extract by destroyed (35), or restruction to another structure (36) that have less effective on bacteria. Also, results in Figure (3) pointed to the presence of negative correlation coefficient (r=-0.99) between average growth of colonies and concentrations. Besides the hot water extracts were more effective on *Klebsiella sp.* than *E. coli* bacteria, which some studies discussed that as some sort of *Funaria sp.* content materials have higher attraction toward cell wall of some kind of bacteria (23; 37), these materials could be activated by heating before exposure to bacteria. However, most of studies didn't show a true mechanism for these effect on cell wall bacteria. On the other hand, few studies mention it as a result of molecular structure of the compounds in hot water extract have ability to convert or conjucate with compounds of cell wall structure (38), while others referred to it as a result of Synergism between materials in hot water extracts (39). Results also showed that *E. coli* bacteria have variety in percentage growth of colonies on different concentrations where the LC_{50} was above 2.5 (the higher concentration that used) with negative correlation coefficient (r=-99) with concentration. Which may be returned to active materials or compounds that could be dissolved in hot water and undestroyed by heat (40).

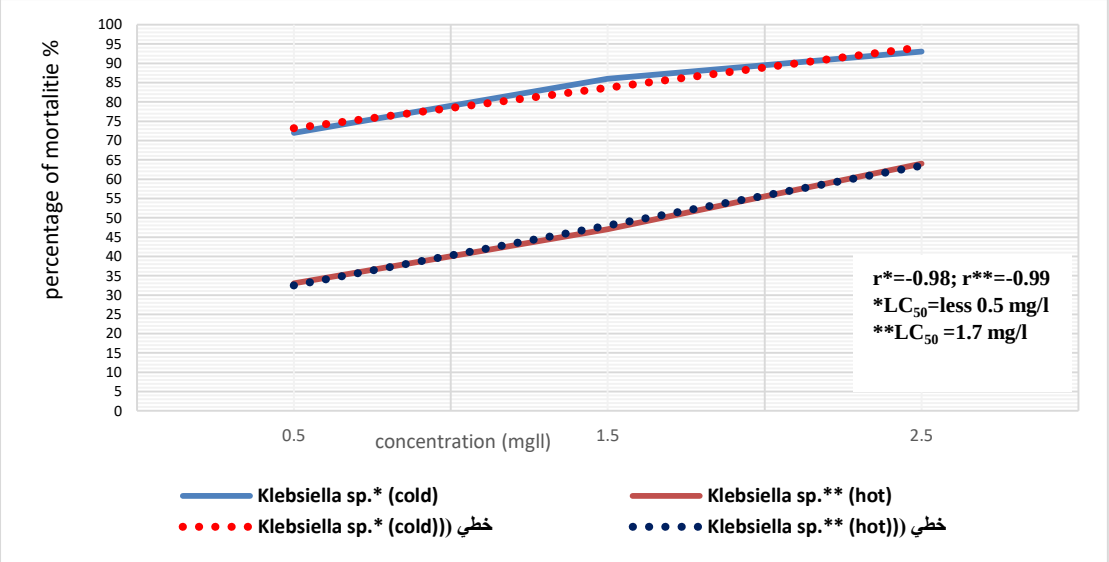

Figure (3) value of LC_{50} after 24 hours of treatment *Klebsiella sp.* bacteria with cold and hot water extracts of *Funaria sp.*

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