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Antimicrobial Activity of Lepidium Sativum against some Gram Positive and Gram Negative Bacteria and Fungi

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Abstract: ***Nowadays it has been wonder if alternative medicine such as using herbs and plants directly could be considered in order to avoid pharmaceuticals enters human body giving side effects such as resistance and allergy rather than treating the local illness. Lepidium Sativum is one of the considerable herbs easy and distributed around different continents regardless the phase of using, some use leaf parts, while as treatment seeds have been used always, hall grains or extracts. This study aimed to distinguish that effect of seeds extracts on some microorganisms and possibility of absolute concentration could achieve that. Using standard organisms, such as E. coli, K. pneumoniae, salmonella para typhi, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa and Candida albicans, inoculated them in different concentrations of the extract, using different solutions base, such as water, crude, hexane, chloroform and ethanol, high sensitivity determined by the size of the zone in the certain dilution, which presented in plate of each plate cultured, was in 50 mg/ml (5%). More effective appeared with E.coli (in crude solution) and K. pneumoniae (in hexane solution) as each zone diameter was 16mm, while the low diameter of sensitivity was obtained with C. albicans with 7mm.***

Key word: ***E. coli, K. pneumoniae, salmonella para typhi, Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Candida albicans, Lepidium Sativum, chloroform and crude***

I. INTRODUCTION

Herbal medicines are the oldest remedies known to mankind. Herbs had been used by all cultures throughout history (1), it has become a popular form of healthcare; although several differences exist between herbal and pharmacological treatments, herbal medicine needs to be tested for efficacy using conventional trial methodology and several specific herbal extracts have been demonstrated to be efficacious for specific conditions (2). Medicinal plant products were proved useful in minimizing the adverse effects of various chemotherapeutic agents as well as in prolonging longevity and attaining positive general health (3). The increasing global interest in the medicinal potential of plants during the last few decades is therefore quite logical (4).

L. sativum (family-Brassicaceae) Fetto (Amharic), Garden cress, (English) is an erect annual herb of up to 60 cm in height. The seeds are rich in minerals and vitamins; especially vitamins C, A, B and E (5). A paste of seed flour mixed with water is used on chapped lips, against sunburn and other skin disorders. Seeds are chewed to cure throat disease, asthma and headache. It is an important spice and medicinal plant which is scattered into and grown with other crops, particularly teff in Ethiopia. More uses of L. sativum in Ethiopia: for colic, abdominal pain, dysentery, swellings, and aphrodisiac (6). It is still popular and frequentlyconsumed type of leafy vegetables in Europe,in Scandinavia, Netherlands, England andFrance (7-8). The seeds are used to treat a variety of skin complaints (9), cold, headache, asthma, sore throat and cough; L. sativum is a source of folic acid,vitamins C, dietary fiber, iron, calcium, protein,vitamin A, folate and vitamin E. The seeds are also highly nutritive and they contain ascorbicacid, tocopherol, folic acid, calcium, linoleic fatty acids and iron (10-11).

II. MATERIALS AND METHODS

The current is experimental study carried out in order to assess antimicrobial activities of L sativum using different microorganisms to do so in several steps.

A. Fresh seeds of Lepidium Sativum

Obtained from an apothecary shop in Omdurman market. The seeds were stored in the air tight container before the preparation of extracts, which performed as: washed seeds of Lepidium sativum with distilled water and then blended using mortar and pestle. Grinded seeds then added to 100 ml of 100% solvents (ethanol, hexane, chloroform, water). The suspension was filtered using Whatman No. 1 filter paper. The filtrate was evaporated and powdered form was obtained. Minimum amount of Dimethyl Sulfoxide

(DMSO) was added in the above obtained powdered extract. Different dilutions of the extract were prepared for antimicrobial assay; dilutions were conducted from crude, water residue, hexane, chloroform and ethanol as 100 (10%), 50 (5%), 25 (2.5%), 12.5 (1.25%), 6.25 (0.6%), 3.12 (0.3%), 1.56 (0.2%) and 0.78 (0.08%) mg/ml, those dilutions for minimum inhibitory concentration (MIC), which assessed visually depending in color changes. And minimum bacterial concentration (MBC) it conducted by sub-culture of each extract dilution and determined by which no growth (blue color) on plate that contained Mueller Hinton agar, MBC is the concentration that inhibited growth f the organism.

B. Microorganisms Used For Antimicrobial Assay

Escherichia coli (ATCC No. 25922), Staphylococcus aureus (ATCC No. 25923), Enterococcus faecalis (ATCC No. 29212) Pseudomonas aeruginosa (ATCC No. 27853), and Klebsiella pneumoniae (ATCC No. 13883); while Salmonella paratyphi B (from stool sample) and Candida albicans (from urine sample) were clinical isolated organisms. Cultures used for antimicrobial assay were obtained from Khartoum National Health Laboratory. Microbial inoculums was be standardized at 0.5 McFarland standards.

C. Agar Well Diffusion Assay

molar Hinton agar was the base of the culture, inoculation of the microorganisms took place first, then using metal porer, different pores were conducted to enable filling with exact extract dilution to be added, which was 50microliter in each pore from each solvent. Antimicrobial activity of plant extracts was screened against E. coli, S. aureus, E. faecalis, P. aeruginosa, K. pneumoniae, S. paratyphi B and C. albicans using the agar well diffusion assay. Microbial inoculum was aseptically spread on the surface of pre solidified Mueller Hinton agar plates using a spreader.

II. RESULT

This experimental study conducted on L. sativum effect on certain microorganisms, as it used as base of growth media, antimicrobial activity can be presented with increasing the zone of clearance, which indicate the effect occurred though, as diameter more than 12 mm would be considered as a sensitive. Five different solutions were used as solvents for the extract of L. sativum seeds, crude, water residue, Hexane, chloroform and ethanol, with different concentrations of seeds, the result showed that more effective readings or growth was obtained through the dilution 50 mg/ml, which revealed different zones in cultured media with different organisms, high zone diameters obtained with E. Coli and K. Pneumoniae as 16mm, and then decline zone diameters until 7mm which obtain by the C. albicans, as in table 1

Table 1:Antimicrobial activity of seeds of Lepidium sativum extracts

Extract	S. aureus	E. faecalis	E. coli	K. pneumoniae	P. aeruginosa	S. paratyphi	C. albicans
Crude	10	13	16	11	9	8	-
Water	12	-	15	9	8	10	12
Hexane	11	12	14	16	13	12	7
Chloroform	15	11	-	12	14	11	12
Ethanol	14	8	11	11	13	-	-

Minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) for each microorganism as in table 2

Table 2: MIC and MBC among different dilutions

Extract	S. aureus		E. faecalis		E. coli		K. pneumoniae		P. aeruginosa		S. paratyphi		C. albicans	
	MIC	MB	MIC	MB	MIC	MB	MIC	MB	MIC	MB	MIC	MB	MIC	MB

		C		C		C		C		C		C		C
Crude	12.5	25	3.12	25	25	50	50	NIL	25	NIL	50	NIL	NIL	NIL
Water	25	50	NIL	NIL	3.12	6.25	12.5	25	NIL	NIL	25	50	12.5	50
Hexane	50	Nil	50	Nil	12.5	25	3.25	12.5	12.5	50	6.25	25	50	50
Chloroform	3.12	12.5	12.5	50	Nil	Nil	25	50	6.25	25	12.5	50	25	50
Ethanol	3.12	6.25	6.25	25	6.25	12.5	25	Nil	3.12	12.5	Nil	Nil	Nil	Nil

III. DISCUSSION

Bacterial resistance to antibiotics has been a recognized and dangerous, resistant strains occurred with a disturbing regularity (12), leading increased death rate (13) and high cost due to unnecessarily prescribed antibiotics (14-15). Thus effective antimicrobials were no longer available which could cure virtually all bacterial infections. This optimism was shaken further by the emergence of resistance to multiple antibiotics amidst enteric pathogens, Pseudomonas aeruginosa, Streptococcus pneumonia, S. aureus and Mycobacterium tuberculosis (16-14-18). It has been searching of some novel antimicrobial molecules which have a broad spectrum of activity against both bacteria without having many or any side effects and exploring the variety of medicinal plants (19). Medicinal plants have continued to receive a lot of attention from researchers due to their pharmacological effects such as anti-inflammatory and antibacterial properties (20). In this study, the aim was to ensure about the effectiveness of garden cress a k a L. sativum on most popular bacteria in the community with recurrent of infection leading to the concept that they were resistant to prescribed medications, so focusing of E. coli, S. aureus, E. faecalis, K. pneumoniae, P. aeruginosa, S. paratyphi and a fungal C. albicans. Using seeds of L. sativum extract diluted in several concentrations, using different solvents, and off course culturing in Mueller Hinton agar as a base media. High sensitivity determined with clear zone diameters, it presented with E. coli in crude and k. pneumoniae in hexane with 16mm, while 15mm in plate of S. aureus in chloroform and E. coli in water dilution, while 14 mm by s. aureus in ethanol dilution, E. coli in hexane dilution and P. aeruginosa in chloroform, 13 mm by E. faecalis in crude and P. aeruginosa in hexane. Candida albicans has 12mm for growth in both water and chloroform. MIC and MBC for each organism were ranged from 50 to nill. Many studies concerned about L. sativum effect on microorganisms, one of the conducted in Sudan as well, it has a partial agreement with this study, as they both conducted on mutual organisms included E. coli,P. aeruginosa and candida albicans, sensitivity presented at concentration of extract (2.5%) 2.5mg/ml while in this study it was on (5%)50mg/ml (21). While other Iraqis study conducted assessing antibacterial effect of ethanolic and aqueous extracts of Lepidiumsativum, also has a partial agreement with this study, it investigated on Gram negativeand Gram positive bacteria (Klebsiella pneumoniae, Proteus, Pseudomonas aeruginosa, Staphylococcus aureus,and Streptococcus mutans). All bacteria under this study were obtained from human infections from by using the well diffusion technique. It was observed that the extracts ofthe plant had an inhibitory effect on all the bacteria under study, except Klebsiella pneumoniae. The minimum inhibitory concentration (MIC) ofL. sativum extracts was determined and it was 3% for Klebsiella pneumoniae (22). Other study conducted to investigate the chemical composition, antioxidant properties and antibacterial effects of nbutanol extract of Lepidium sativum seed. Theantimicrobial activity of the extract was tested against five strains of pathogenic microorganisms, Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 43300, Enterococcus feacalisATCC 29212, Escherichia coli ATCC 25992 and Pseudomonas aeruginosa ATCC 27852. The effect of L. sativum approved that the use of Lepidium sativum in traditional medicine (23).

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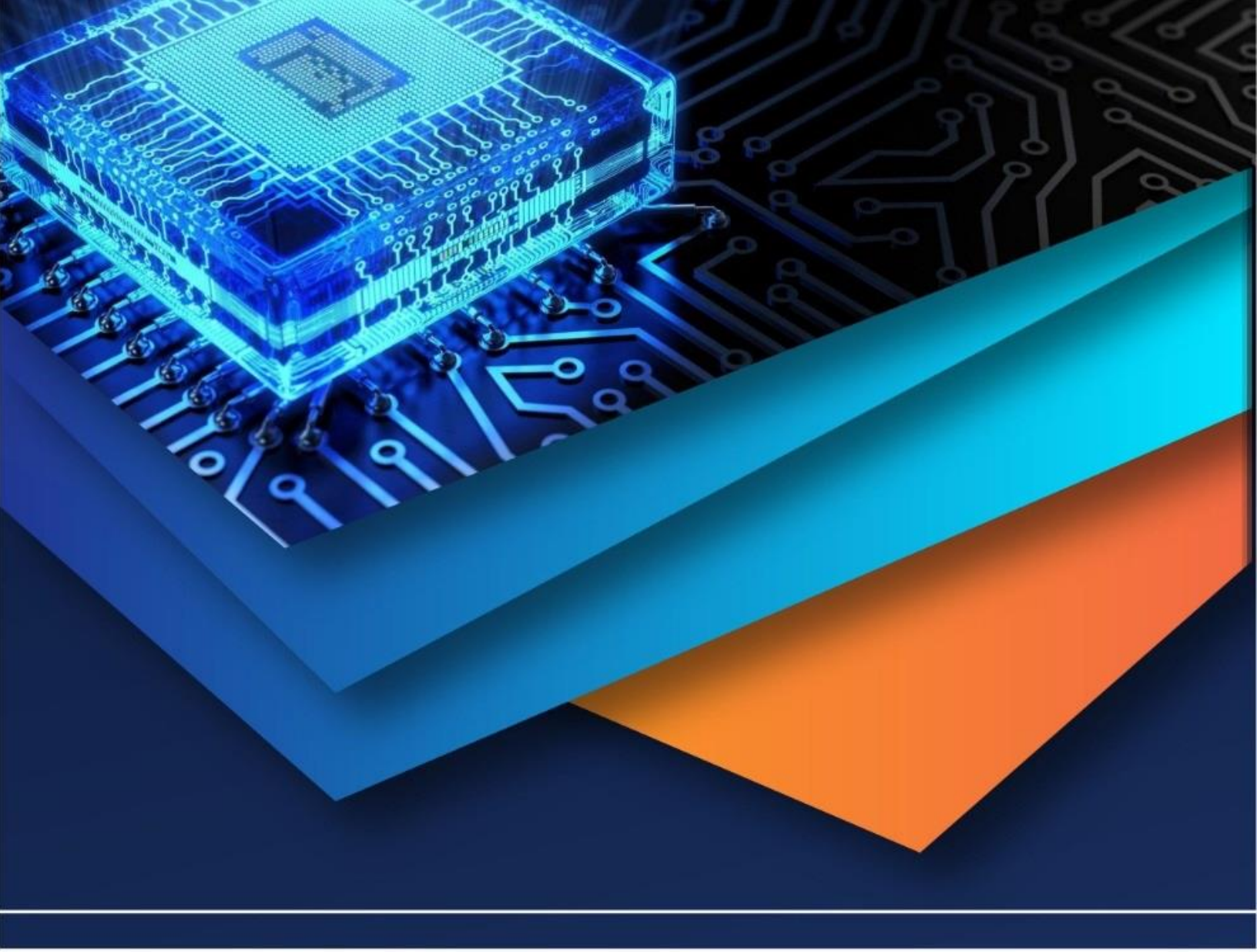

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