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Research Article

An Investigation of Antibacterial Effects of *Hypericum Perforatum L.* Polar, Semi-Polar, and Non-Polar Fractions on a Number of Gram-Positive and Gram-Negative Bacteria

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ABSTRACT

Increased levels of drug resistance among bacteria has caused a new attention to finding methods for prevention of such resistances and also drugs suitable for toxicity and fewer side effects. To do so, recent outlooks are pointed to herbal medicine—or, herbalism. The present study was aimed at determination of antibacterial effects of polar, semi-polar, and non-polar fractions of Hypericum Perforatum on *staphylococcus aureus*, *bacillus cereus*, *Escherichia coli*, and *pseudomonas aeruginosa* bacteria. In this article, extraction of Hypericum Perforatum was used. Firstly, methanol, chloroform, and petroleum extractions of Hypericum Perforatum were prepared and impact of different concentrations was studied. All experiments were conducted by well diffusion method and determination of MIC and MBC on standard strains. Methanol, chloroform, and petroleum extractions of Hypericum Perforatum had the highest impact on the gram-positive bacillus cereus bacterium. While, higher concentrations are required for impacts on gram-negative *Escherichia coli* and *pseudomonas aeruginosa* bacteria. Methanol, chloroform, and petroleum extractions of Hypericum Perforatum had considerable impacts on *bacillus cereus*, *staphylococcus aureus*, and *pseudomonas aeruginosa* bacteria. In the meanwhile, Escherichia coli were a resistant bacterium against all three tested fractions.

Keywords: Hypericum Perforatum L.; *Bacillus cereus*; Herbal extraction; *Escherichia coli*

1. INTRODUCTION

Increased levels of drug resistance among bacteria has caused a new, higher attention to finding methods for prevention of such resistances and also drugs suitable for toxicity and fewer side effects. To do so, recent heeds are paid to herbal medicine—or, herbalism [2]. Application of herbal medicine to treat human illnesses represents a long history. It is estimated that more than 10 percent of known plant species come with medicinal use. A WHO (World Health Organization) study estimates that around 80

percent of world population makes use of herbal medicine for healthcare purposes. Given the fact that herbal medicines are widely distributed in our country, investigation of such plants regarding their antibacterial features may herald the replacement of natural medications to control and treat bacterial infections. This makes a reduction in consumption of chemical drugs and prevents their ensuing complications. Due to their relative healthfulness and popularity, essential oils and their combinations are under the focus of many researchers. Antibacterial, antifungal, and antioxidant properties of many essential oils are examined by several scholars.

Referred to in English as Goat Weed or St John's wort, *Hypericum Perforatum* is an invaluable, herbaceous perennial plant from the hypericaceae family. It grows in sunny areas equipped with sandy soil and fine drainage conditions. Typically, *Hypericum Perforatum* rises up on roadsides and along railways. *Hypericum Perforatum* has numerous combinations with biological activities such as naphthodianthrones, flavonoids, xanthenes, proanthocyanidins, bioflavonoids, phloroglucinols, essential oils, and derivatives of amino acid and phenyl propane. Anabolic, anti-inflammatory, anti-bacterial, anti-viral, protein-making, and other impacts by *Hypericum Perforatum* are mostly because of flavonoids existing therein. Due to existence of hyperforin, *Hypericum Perforatum* has cell toxicity impacts against carcinoma of colon and anti-bacterial effects especially against *staphylococcus aureus*. Herbal flavonoids are anti-inflammatory, anti-ulcer, sedative of central nervous system, and anti-virus (including hepatitis c and HIV) [4].

Staphylococcus aureus is one of the most important factors for hospital infections. One of the success factors of this bacterium is fulfillment of resistance factors, in which process resistant bacterium strains quickly appear and make difficult treatment of infections ensuing from such bacteria after new antibiotics are entered [5].

Escherichia coli is a natural flavonoid in the intestine of all warm-blooded animals. The number of these bacteria is higher in intestine of carnivores, humans, and monkeys than in that of herbivores. *Escherichia coli* is thus a factor of infections in foods like milk. This bacterium causes bloody diarrhea and hemolytic uremic syndrome in humans [3].

Pseudomonas aeruginosa bacterium is the most common human pathogen. This is the agent of the most hospital infection. *Pseudomonas aeruginosa* is an important pathogen which leads to death in many patients of cystic fibrosis and serious burns [10].

There are studies carried out on the anti-bacterial effects of *Hypericum Perforatum* in the world and Iranian markets. Little, however, is the research about anti-bacterial properties of polar, semi-polar, and non-polar fractions of this plant. This study, accordingly, intends to investigate anti-bacterial properties of polar, semi-polar, and non-polar fractions of *Hypericum Perforatum* on *staphylococcus aureus*, *bacillus cereus, Escherichia coli,* and *pseudomonas aeruginosa* bacteria.

2. MATERIALS AND METHODS

2.1. Bacterial strains

Bacterial strains used in this study are shown in the Table 1 below:

Table 1: Bacterial strains

No.	Name of strain
1	*Staphylococcus aureus* PTCC/1112
2	*Escherichia coli* PTTCC/1270
3	*Pseudomonas aeruginosa* PTCC/1430
4	*Bacillus cereus* PTCC/1015

2.2. Reduction of Bacterial Strains

Microorganisms used herein were *staphylococcus aureus, bacillus cereus, Escherichia coli,* and *pseudomonas aeruginosa* which were conferred in Lyophilized mode by Tehran University Biotechnological Research Center. Bacterial samples were reduced as per standard reduction methods. Since the number of inoculated bacteria is one of the most important variables that impacts on study's results, concentration of inoculated bacterial suspension should be standard. To prepare bacterial suspension from young cultivation, multi-colon bacterium was trans-

planted to Mueller Hinton Broth cultivation environment. It was, then, incubated for 2 hours at 37° C so as to make its opacity similar to 0.5 McFarland standards (1.5×108 per milliliter). Bacterial suspension was, afterwards, diluted to proportion of 0.01 in order to reach 1.5×106 bacteria per milliliter.

2.3. Preparation of *Hypericum Perforatum* Extraction

In this research, categorization of fractions was conducted based on polarity of applied solvents. To do so, herbal materials were, firstly, extracted by a non-polar solvent such as petroleum. Obtained extraction was purged from solvents, followed by the next stage where materials remained form the previous process were re-extracted using less polar solvents such as chloroform. Finally, remained herbal materials were extracted using more polar solvents such as methanol. This resulted in three categories of extractions which were different in their polarity. This method has been adopted more in pharmacognosy research.

2.4.Conduction of the Experiment

From among the extractions obtained by 5% dimethyl sulfoxide (DMSO) solvent, densities of 0.39, 0.78, 12.56, 6.25, 25.5, 50, and 100 milligrams per milliliter were used to be applied in well diffusion test and determine MIC and MBC. In well diffusion test, 500 microliters of bacterial suspension with 1.5×106 cfu/ml was uniformly developed in Mueller Hinton Agar cultivation environment. Then, wells with diameters of 5 milliliters were, in plate level, separated from each other in 2.5 cm spans. 100 microliters of each density were transplanted into wells. DMSO is regarded as the negative observer and chloramphenicol and tetracycline antibiotics as positive ones. After its completion, all incubated cultivation environments were placed in incubator at 37° C for 24 hours. After a determined duration of time, bacterial cultivations were checked for formation/non-formation of halos around wells, and diameter of non-formation halo was measured in terms of millimeters. Diameter of halos is a reaction of tested extraction density. This phenomenon is a linear relationship between halo and logarithm of tested density of extraction, whose antibacterial power was determined through measurement of halo's diameter of non-growth and its comparison with certain standards.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined using the Microtiter Plate-based Method through resazurin test. In this method, 96-cell microplates of cells 1 to 9 represent densities of 39% to 100% milligram in milliliter. Cell 10 represented bacterium, cell 11 environment, and cell 12 extraction.

1st stage: In all cells, save for the first cell, 100 ml of Mueller Hinton Broth cultivation environment was allocated.

2nd stage: 100 ml of prepared extraction was poured in first and second cells in order to make 100% the density of the first cell. Then, 100 ml of extraction was removed from the second cell to the third one, from the third to the fourth, and so on to the ninth cell. 0 ml of extraction was removed from the ninth cell, finally.

3rd stage: After 24 hours of bacterial cultivation, 1.5×108 cfu/ml of 0.5 McFarland opacity (1 to 100) was prepared and 100 microliter of it was poured in all cells.

4th stage: 10 ml of resazurin assay was poured into all cells.

Above method was applied for polar, semi-polar, and non-polar extractions.

After incubation, resazurin dye change of cells from blue-violet to pink as a result of incubated bacteria was checked. The least opacity of the extraction in which no dye change was observed was selected as MIC. To determine MBC, all tubes which had not been grown were cultivated in Mueller Hinton Agar cultivation environment. Inoculated cultivation environments were incubated for 24 hours at 37° C. The plate which included the least extraction density and was devoid of bacterial growth was selected as MBC. All above-cited stages were applied for polar, semi-polar, and non-polar extractions.

3. RESULTS

Results of the findings on impact of different concentrations on selected bacteria are exhibited in Tables 2, 3, and 4.

Comparison among different petroleum, chloroform, and methanol concentrations of *Hypericum Perforatum* showed that bacillus cereus bacterium and *Escherichia coli* bacterium are the most sensitive and resistant bacteria, respectively.

Table 2: Impact of petroleum extraction concentrations of *Hypericum Perforatum* by well diffusion test and determination of MIC/MBC

Bacteria	Well	1	2	3	4	5	6	7	8	9	10	11	12	C 30mcg		TE 30mcg	
	%	100 %	50 %	25 %	12.5 %	6.25 %	3.125 %	1.56 %	0.78 %	0.39 %	Bacterial Control	Control	Extract Control	mm	Zone	mm	Zone
Escherichia coli PTTCC -1270	MIC	-	-	-	+	+	+	+	+	+	+	–	–	23	S	15	i
	MBC	-	-	-	+	+	+	+	+	+							
	Well	0	0	0	0	0	0	0	0	0							
Staphylococcus aureus PTCC-1112	MIC	-	-	-	-	+	+	+	+	+	+	–	–	24	S	23	S
	MBC	-	-	-	+	+	+	+	+	+							
	Well	10	8	0	0	0	0	0	0	0							
Bacillus cereus PTTCC -1015	MIC	-	-	-	-	-	-	-	-	+	+	–	–	25	S	30	S
	MBC	-	-	-	-	-	-	-	-	+							
	Well	10	0	0	0	0	0	0	0	0							
Pseudomonas aeruginosa PTCC.1430	MIC	-	-	-	+	+	+	+	+	+	+	–	–	20	S	26	S
	MBC	-	-	-	+	+	+	+	+	+							
	Well	8	0	0	0	0	0	0	0	0							

R=resistant i=semi-sensitive s=sensitive C=chloramphenicol TE=Tetracycline

Table 3: impact of methanol extraction concentrations of *Hypericum Perforatum* by well diffusion test and determination of MIC/MBC

Bacteria	Well	1	2	3	4	5	6	7	8	9	10	11	12	C 30mcg		TE 30mcg	
	%	100 %	50 %	25 %	12.5 %	6.25 %	3.125 %	1.56 %	0.78 %	0.39 %	Bacterial Control	Control	Extract Control	mm	Zone	mm	Zone
Escherichia coli PTTCC -1270	MIC	-	-	-	+	+	+	+	+	+	+	-	-	23	S	15	i
	MBC	-	-	-	+	+	+	+	+	+							
	Well	0	0	0	0	0	0	0	0	0							
Staphylococcus aureus PTCC-1112	MIC	-	-	-	-	+	+	+	+	+	+	-	-	24	S	23	S
	MBC	-	-	-	+	+	+	+	+	+							
	Well	22	18	15	6	0	0	0	0	0							
Bacillus cereus PTTCC -1015	MIC	-	-	-	-	-	-	-	-	+	+	-	-	25	S	30	S
	MBC	-	-	-	-	-	-	-	-	+							
	Well	14	0	0	0	0	0	0	0	0							
Pseudomonas aeruginosa PTCC.1430	MIC	-	-	-	+	+	+	+	+	+	+	-	-	20	S	26	S
	MBC	-	-	-	+	+	+	+	+	+							
	Well	14	12	7	0	0	0	0	0	0							

R=resistant i=semi-sensitive s=sensitive C=chloramphenicol TE=Tetracycline

Table 4: impact of chloroform extraction concentrations of *Hypericum Perforatum* by well diffusion test and determination of MIC/MBC

Bacteria	Well	1	2	3	4	5	6	7	8	9	10	11	12	C 30mcg		TE 30mcg	
	%	100 %	50 %	25 %	12.5 %	6.25 %	3.125 %	1.56 %	0.78 %	0.39 %	Bacterial Control	Control	Extract Control	mm	Zone	mm	Zone
Escherichia coli PTTCC -1270	MIC	-	-	-	+	+	+	+	+	+	+	-	-	23	S	15	i
	MBC	-	-	-	+	+	+	+	+	+							
	Well	0	0	0	0	0	0	0	0	0							
Staphylococcus aureus PTCC-1112	MIC	-	-	-	-	+	+	+	+	+	+	-	-	24	S	23	S
	MBC	-	-	-	+	+	+	+	+	+							
	Well	15	10	8	0	0	0	0	0	0							
Bacillus cereus PTTCC -1015	MIC	-	-	-	-	-	-	-	-	+	+	-	-	25	S	30	S
	MBC	-	-	-	-	-	-	-	-	+							
	Well	12	0	0	0	0	0	0	0	0							
Pseudomonas aeruginosa PTCC.1430	MIC	-	-	-	+	+	+	+	+	+	+	-	-	20	S	26	S
	MBC	-	-	-	+	+	+	+	+	+							
	Well	12	10	5	0	0	0	0	0	0							

R=resistant i=semi-sensitive s=sensitive C=chloramphenicol TE=Tetracycline

4. DISCUSSION

Antibacterial combinations obtained from plants with mechanism different from antibiotics are able to omit bacteria, the fact which is clinically of paramount importance in treatment of infections ensuing from resistant bacterial strains. With respect to a revival of herbal medicine, investigation of medicinal properties of plants has gained a new significance. After investigation of results, it is understood that petroleum extraction of investigated plant has higher impacts on gram-positive bacteria rather than gram-negative ones. This impact is more on bacillus cereus than others, and *Escherichia coli* was wholly resistant against this combination. MIC and MBC amounts were 0.78 for this bacterium.

Results of impact of chloroform extraction show that *staphylococcus aureus* and *Escherichia coli* are, respectively, the most sensitive and resistant bacteria in this combination.

Results of methanol extraction indicate that this extraction, at its 12.5 microgram density, prevents gram-positive *staphylococcus aureus* bacterium development. While higher densities are required for leaving impacts on gram-negative *Escherichia coli* and *pseudomonas aeruginosa* bacteria. These results are indicative of anti-bacterial impacts of methanol extraction of *Hypericum Perforatum* against gram-positive bacteria; whereas, it has impotent preventive impacts on gram-negative bacteria. This is possibly due to the fact that lipopolysaccharides of cell walls prevent extraction combinations from reaching at cytoplasmic membrane of gram-negative bacterium. Since most compositions in extractions come with hydrophobic nature, this is safe to say that these materials are not able to infiltrate and get access to the points activated in gram-negative bacteria. Generally, herbal products cause granulation of cytoplasm, disruption of cytoplasmic membrane, inactivation or inhibition of intracellular and extracellular enzyme activities,

and collapse of cell walls so that most herbal extractions have preventive effects on gram-positive bacteria and limited impacts on gram-negative bacteria.

In their article entitled "Investigation of Antibacterial Impacts and a Review of Ten Plant Species against Nocardia Pathogenic Strains," Eshraghi et al. (2009) drew the conclusion that extraction of *Hypericum Perforatum* with density of 15% has inhibitive effects on Nocardia asteroids similar to amikacin, amoxicillin, and ceftazidime antibiotics. In this study, effects of different fractions of *Hypericum Perforatum* on other pathogenic bacteria were investigated, and results indicated existence of antibacterial properties [1].

Radulovich et al. (2007) presented an article entitled "Identification of Antioxidant and Antibacterial Activities of Nine Species of *Hypericum Perforatum* under Laboratorial Conditions," which was expressive of potent antibacterial properties against bacteria under the investigation. Results of this study and those made herein about antibacterial properties of *Hypericum Perforatum* are aligned [8].

In their study, Mashreghi et al. (2012) stated that extraction of *Hypericum Perforatum* prevents bacterium development phases of E. coli O157. Impact of *Hypericum Perforatum* extraction on E. coli bacterium has been proved in both studies [6].

Meral et al. (20020 showed that extraction of three species of *Hypericum Perforatum* has high antibacterial properties [7].

Saddige et al. (2010) expressed in their article that *Hypericum Perforatum* extraction has bactericidal properties [9].

5. CONCLUSIONS

In this study, it was specified that methanol and chloroform extractions of *Hypericum Perforatum* have considerable preventive impacts of the bacteria under investigation. Results of antibacterial tests on methanol and chloroform extractions of *Hypericum Perforatum* indicate some impacts on gram-negative and gram-positive bacteria so that preventive impact of methanol and chloroform extractions on bacillus cereus, as a gram-positive bacterium, is higher than other three bacteria. This is attributed to different reasons including structural differences in walls of these bacteria and/or their innate resistance due to different mutations and recombination.

6. Suggestions

This is suggested to other researchers to study and identify effective compositions of *Hypericum Perforatum* that have antibacterial impacts. In addition, antibacterial impacts of *Hypericum Perforatum* on food material bacteria can be studies. Impact of *Hypericum Perforatum* extraction on resistant strains and its application within living organisms may constitute the subject of another research.

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