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THE BIOLOGICAL ACTIVITIES OF *HYPERICUM PERFORATUM* L.

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Abstract

Background: Mastitis reduces milk yield and alters milk composition. Antibiotics are widely used in the treatment of the disease. However, this widespread use of antibiotics causes both antibiotic residues in milks and antibiotic resistance developed in bacteria. Today's researches are focused on discovering and using new antibiotics against bacteria.
Objective: The aim of this work was to discover the antibacterial effects of *Hypericum perforatum* L. extracts against mastitis pathogens, and its other biological activities.
Material and Methods: Kirby-Bauer assay was applied to the extracts. The other antibacterial activity was MIC for plant extracts. The non-enzymatic antioxidant activity was found using 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH).
Results: The extract was showed maximum inhibition zone against two bacteria (Coagulase-negative *Staphylococci*-33 and 37; CNS 33 and 37), and the zone was 17 mm. A bacterium (CNS – 22) showed the lowest sensitivity to 812.5 µg /mL concentration. In addition, the extract was tried against the stable DPPH for antioxidant activity. As a result, the extract showed a strength antioxidant activity. Trolox equivalent is 0.83 mM.
Conclusion: The extract of *Hypericum perforatum* have antibacterial, antioxidant and antimutagenic potentials.

Keywords: *Hypericum*, Mastitis, Antibacterial activity, Antioxidant activity, Antimutagenic activity

Introduction

Mastitis is a complex disease, which is defined as inflammation of parenchyma of mammary glands and is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues (Radostits *et al*., 2000). The widespread causative organisms of breast disease include: *Staphylococcus*, *Streptococcus* and coliform bacteria (McDonald, 1979). Coagulase-negative *staphylococci* (CNS) have been considered to be minor mastitis pathogens, especially in comparison with major pathogens such as *Staphylococcus aureus*. The main reason for this is that mastitis caused by CNS is very mild, and usually remains subclinical (Taponen *et al*., 2006). The significance of CNS, however, needs to be reconsidered as in many countries they have become the most common mastitis-causing agents (Pitkala *et al*., 2004; Tenhagen *et al*., 2006).

Studies on medicinal plants are rapidly increasing because of the search for new active substances, and for the improvement in the production of plants or molecules for herbal pharmaceutical industries (Poutaraud *et al*., 2005). World Health Organization reported that, medicinal plants are the best source of drugs. However, these plants must be investigated for their salient features, safety and effects (Nascimento *et al*., 2000).

Hypericum perforatum L. (St. John's wort) is a well-known medicinal plant that has been in use for a decade (Gadzovska-Simic, 2012). This plant is a representative of the Hypericaceae family with confirmed therapeutic effects on burns, bruises, swelling, anxiety, mild to moderate depression (Luo, 2004), antidepressant, antiviral, wound healing, analgesic, hepatoprotective, antioxidant and antimicrobial activity (Jakovljevic, 2000; Popovic, 2002; Radulovic, 2007; Spiteller, 2008). Recently, the antiviral and antidepressant properties of *Hypericum* have become widely demonstrated (Luo, 2004). Extracts from *H. perforatum* are known to contain compounds from six major natural product groups: naphtodianthrones, acylphloroglucinols, flavonol glycosides, biflavones, proanthocyanidins, and phenylpropanes (Nahrstedt and Butterweck 1997). A lot of plants have been used due to their biological activities. Because, these plants synthesize sekonder substances. The biological activities of *Hypericum perforatum* flower extracts against mastitis bacteria have not been studied. Additionally, antioxidant activities of *H. perforatum* flower extracts is less studied comparing to its other biological impacts. In our work, the methanol extract of flower was investigated for antibacterial, non-enzymatic antioxidant and antimutagenic effects.

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Material and Methods

Plant Material

Hypericum perforatum flowers were picked up from different locations of Selcuk, Izmir region, Turkey in 2014. Taxonomical identification of plant was performed by Dr. Olcay Ceylan from the University of Mugla Sitki Kocman, Turkey and a specimen was stored in the herbarium. The identity of these specimen was applied by the Flora of Turkey (Davis, 1965).

The flowers were cleaned thoroughly 2-3 times with flowing and sterile distilled water. Green flower material was dried by air. These flowers pulverized in a disruptive mill. All of samples were stocked at room temperature. Then the flowers were stocked at 4°C until needed for assay.

Plant Extraction

The dried materials (30 g) were extracted with organic solvent (methanol) by the Soxhlet apparatus. These materials were evaporated. Then materials were stored in mat bottles. These vials were stored refrigerated until use (100 mg/mL).

Microorganisms and Cultivation

In this work, *Hypericum perforatum* flowers extracts were singly tried against mastitis bacteria. These pathogens provided from previous studies by Dr. Zafer Cantekin, Mustafa Kemal University, TURKEY (Project number: 1101 M 0103; Ethics council number: 2010 / 02- 30: 12). We have been used two *S. aureus* and five coagulase- negative *staphylococci* (CNS) in our study. These pathogens were grown at 37 °C for 24 hour. We were used Mueller - Hinton Broth (MHA) for cultivation of bacteria. All of bacteria were identified by traditional biochemical tests (Quinn, 1994).

Antibacterial Activity Assay

In this study, Kirby – Bauer assay was applied for antibacterial activities. The bacteria were preserved on MHA plates at 37 °C (Bauer, 1966). Bacterial cultures adjusted to 0.5 Mc Farland. Incubations of bacteria were at 37 °C for 24 hour. The assessment of antibacterial activity was based on measurement of the diameter of the inhibition zones around the discs after 24 hour. We have used methanol and ampicillin (10µg) as control in our study. All tests were performed in triplicate and the mean values were given.

Minimum Inhibitory Concentration Assay (MIC)

The MICs of plant extracts were measured by broth dilution assay. The MIC values were given as the lowest concentration of plant extracts. The broth dilution assay was done according to CLSI standards (CLSI, 2003; CLSI, 2006). We have used final concentrations of the extract in our work. These concentrations were from 6500 µg/mL to 406.25 µg/mL (6500; 3250; 1625, 812.5, and 406.25 µg/mL).

Determination of non-Enzymatic Antioxidant Activity

The non-enzymatic antioxidant activity was decided using DPPH free radical. The stable DPPH was used for determination of free radical-scavenging activity of the flower extract. Extract (0.1 mL) was added to methanol DPPH mixture (0.1 mM). This solution was incubated for 30 minutes and after, absorbance of solution was measured spectrophotometrically. Blank was methanol. We have used methanol with DPPH solution as control (Brand-Williams, 1995). Additionally trolox was used for reference. The free radical scavenging capacity expressed in percentage (%) was calculated from formula.

Antimutagenic Activity Assay

Antimutagenic activities of extracts were done by the *Salmonella*-microsome assay. In this study, *Salmonella* Typhimurium tester strains were used for antimutagenic activity tests. These bacteria were provided from previous studies by Dr. B.N. Ames (Maron and Ames, 1983). In our study, two tester strains were utilized to test the antimutagenicity of *H. perforatum* extract. These bacteria are *Salmonella* Typhimurium TA98 and TA100. The calculation percentage of antimutagenic activity was done according to Ong *et al*. (1986). Sodium azide was used as positive control. Methanol is negative control. Concurrently, a positive control (with mutagen but no extract was added) and a negative control (where no mutagen was added) were also set. The test sample was dissolved in methanol. But mutagen was dissolved in distilled water. In our study, non-toxic concentrations of the test sample used for investigating were 12500 and 25000 µg /plate. These concentrations were categorized as non-toxic because they

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showed a well-developed lawn, almost similar size of colonies and no statistical difference in the number of spontaneous revertants in test and control plates.

Results

The antibacterial activities of flower extracts were tried against mastitis bacteria. Results of this study are given in Table 1. The results of inhibition zones were recorded as mm. The extracts suppressed the growth of bacteria. These inhibition zones formed against bacteria ranged between 13- 17 mm. *Hypericum perforatum* extracts have been quite effective against 2 bacteria. The highest inhibition zone was determined in CNS-33 and 37, and its zone was 17 mm. Ampicillin (10µg) is positive control. Ampicillin very strongly supressed the growth of *S. aureus*- 17 (Table 1).

Table 1: Antibacterial activities of *Hypericum perforatum* flower extracts

Bacteria	Inhibition zone diameters (mm)	Ampicillin
S. aureus- 17	13	18
S. aureus- 18	16	12
CNS - 22	16	-
CNS - 32	16	10
CNS - 33	17	8
CNS - 36	16	-
CNS - 37	17	-

CNS: Coagulase Negative *Staphylococci*

Table 2 shows MICs of *H. perforatum* obtained by the broth dilution method. CNS-22 demonstrated the lowest susceptibility to methanol extract (812.5 µg /mL) (Table 2).

Table 2: Minimum inhibitory concentrations of *Hypericum perforatum* flower extracts

Bacteria	Minimum inhibitory concentration (methanol; µg/mL)
S. aureus- 17	3250
S. aureus- 18	1625
CNS – 22	812.5
CNS – 32	1625
CNS – 33	1625
CNS – 36	1625
CNS – 37	1625

CNS: Coagulase Negative *Staphylococci*

DPPH radical scavenging capacity was used for antioxidant activities of the flower extracts. The antioxidant activities of flower extracts shown in Table 3. The extract showed 32 % inhibition at 100 mg/mL extract concentration. Trolox equivalent value was 0.83 mM/g DW (Table 3).

Table 3: DPPH radical scavenging capacity of *H. perforatum* flower extract

Flower extracts	DPPH (%)	Trolox equivalent (mM/gDW)
Methanol	32	0.83

DW: dry weight

The antimutagenic capacities of *H. perforatum* flowers are given in Table 4 and 5. In this work, to determine the antimutagenic activity of *H. perforatum*, diagnostic mutagens were inoculated in the sample petri dishes, and two strains were used to measure this activity. *H. perforatum* flower extract (25000 µg /plate) calculated a strong positive inhibitory influence for *S.* Typhimurium TA98 (64.6%) and *S.* Typhimurium TA100 (49.7%) (Table 4).

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Table 4: Antimutagenic activities of *H. perforatum* flower extracts (25000 µg /plate)

Treatment	*Salmonella* Typhimurium TA98		*Salmonella* Typhimurium TA100	
	Number of revertants	% Inhibition	Number of revertants	% Inhibition
Control	42		84	
Negative control (methanol)	43		87	
Pozitive control (sodium azid)	113		161	
H. perforatum extract	40	% 64.6	81	% 49.7

A moderate inhibitory impact (39%) for *S.* Typhimurium TA 100 was determined from *H. perforatum* flower extract (12500 µg /plate). Whereas *H. perforatum* flower extract (12500 µg /plate) showed strong inhibitive effect (58%) for *S.* Typhimurium TA98 (Table 5).

Table 5: Antimutagenic activities of *H. perforatum* flower extracts (12500 µg /plate)

Treatment	*Salmonella* Typhimurium TA98		*Salmonella* Typhimurium TA100	
	Number of revertants	% Inhibition	Number of revertants	% Inhibition
Control	42		84	
Negative control (methanol)	43		87	
Pozitive control (sodium azid)	113		161	
H. perforatum extract	47	% 58.4	98	% 39.1

Discussion

Plant oils and extracts have been used for a wide variety of purposes for many thousands of years (Jones, 1996). Scientists proved that medicinal plants have rich sources of biologically active compounds. Many of these materials have been used as compounds to improve novel medicinal drugs (Palombo, 2011). This study confirms that *H. perforatum* flowers possess antibacterial, antioxidant and antimutagenic activities.

In the present study, the methanol extracts were tried against mastitis pathogens. These results were compared with ampicillin. *H. perforatum* flowers demonstrated low impact against a bacterium (Table 1). Meral and Karabay (2002) reported parallel results. This report support the results of our study. In our work, the methanol extracts were found highly effective for six bacteria, except *S. aureus*- 17 (Table 1). *Hypericum* extract demonstrated bactericidal activity *in vitro* against a number of Gram positive and Gram negative bacteria, including *Staphylococcus aureus*, *Proteus vulgaris*, *Escherichia coli* and *Pseudomonas aeruginosa* (Barbagallo and Chisari, 1987). Keleş *et al.*, (2001) found that extract of *H. perforatum* has a broad spectrum of inhibitory activity. Additionally the extract of *H. perforatum* showed an inhibitory effect of 16 and 14 mm against *Staphylococcus aureus*, *Streptococcus agalactiae*, respectively (Keleş *et al*., 2001). The report support the results obtained from our study. Rancic *et al.*, (2005) declared that extract of *H. perforatum* has demonstrated inhibition against *S. aureus* and this zone is 5 mm. Results of our study are better than Rancic's result (2005). It is not surprising that there are differences in the antibacterial effects of plant groups, due to phytochemical differences among species.

The lowest sensitivity to methanol extracts determined on CNS-22 (Table 2). Dordevic *et al*., (2013) reported that minimum inhibitory concentration value (MIC) of *H. annulatum* and *H. elegans* essential oils against S. *aureus* was found as 3.13 mg/mL. In other study, essential oil isolated from the aerial parts of *H. rumeliacum* exhibited moderate activities against all the tested bacteria (*S. aureus*, *S. epidermidis*, *E. coli*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), with a MIC of 3.80–17.20 mg/mL (Couladis *et al*., 2003). In our work, the lowest MIC value of flower extract was determined as 812.5 µg/mL. Our findings are better than MIC results of other scientists (Dordevic *et al.*, 2013; Couladis *et al.*, 2003). Aligiannis et al,. (2001) reported that plant materials were able to be classified based on MIC results. The methanol extracts of *H. perforatum* can be accepted as low inhibitor against mastitis bacteria. Saroglou *et al*., (2007) reported that MIC value of *H. perforatum* was 12.5 µg/mL for *S. aureus*. The reason for this may be the plant composition. Essential oils content and composition can be greatly affected by several parameters including seasonal variation (Guedes *et al*., 2004), phenological cycle (Schwob *et al.*, 2004) and geographic distribution.

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Cai et al., (2004) reported that free radical scavenging abilities of plants are responsible for their curative impact against some diseases. The antioxidant activity of *H. perforatum* is given in Table 3. The flower extract was inhibited free radical and inhibition was determined as 32 % at 100 mg/mL extract concentration. Trolox equivalent value was 0.83 mM/g DW. Wojdylo *et al.*, (2007) reported that antioxidant capacity of *H. perforatum* was found as 82.3 µM trolox/100 g dw. These results of Wojdylo *et al*., (2007) were not as good as our results. Vardapetyan et al., (2014) reported that the highest radical scavenging capacity was found in ethanolic extract of *H. perforatum* leaves. Variations in essential oil composition of several species of the *Hypericum* genus were previously reported, depending on genetic factors (Petrakis *et al*., 2005), geographical distribution and environmental factors (Couladis *et al*., 2001; Smelcerovic *et al*., 2007), ontogeny (Schwob *et al*., 2004; Nogueira *et al*., 2001), and plant organ (Bertoli *et al*., 2003).

In this work, *H. perforatum* flower extract (25000 µg /plate) demonstrated a strong positive inhibition for *Salmonella* Typhimurium TA98 (64.6%) and *S.* Typhimurium TA100 (49.7%) (Table 4). Peron et al., (2013) showed that this plant had no mutagenic potential in subchronic gavage treatments (0.3, 3.0 and 30.0 mg/mL). Ndhlala *et al.*, (2011) determined that *H. aethiopicum* was not mutagenic by the Ames test with *Salmonella* Typhimurium. The reports support the results obtained from this work. In present study, *H. perforatum* flower extract (12500 µg /plate) was given a positive inhibitory impact against *S.* Typhimurium TA98 (58%). In addition, this dose was showed moderate inhibitory effect for *S.* Typhimurium TA100 (39%) (Table 5). An antimutagenic activity was demonstrated by *Hypericum* extract on DNA repair in *Escherichia coli* (Vukovic-Gacic and Simic, 1993). The report support our results obtained from study.

Conclusion

In conclusion, methanol extract of *H. perforatum* tested in the study was determined to have potential antibacterial activities against S. *aureus* and CNS pathogens isolated from subclinical cow mastitis. Our findings recommend that *H. perforatum* has important antibacterial activity. We believe that it could be very beneficial in the field of new antibacterial agents of plant origin. Furthermore, methanol extracts of the plant have less importance as antioxidant activities. Additionally, this plant was showed antimutagenic properties and which is high important. The study suggests that *Hypericum perforatum*, owing to its antimutagenic property, can be beneficial for prevention of cancer. Further research is required to determine the best antioxidant, antimutagenic and antibacterial agents.

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