Research Journal of Agricultural Sciences 9 (2): 51-54, 2016
ISSN: 1308-3945, E-ISSN: 1308-027X, www.nobel.gen.tr

Antibacterial Potential of *Hypericum calycinum* L. from Turkey

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Received: August 17, 2016
Accepted: December 07, 2016

Abstract

In the present study, the antibacterial activity of the crude extracts obtained from flowers, leaves and stems of *Hypericum calycinum* L.against *Streptococcus mitis* CNCTC 4/77, *Streptococcus salivarius* CNCTC 64/59, *Streptococcus mutans* CNCTC 8/77 and *Staphylococcus epidermidis* ATCC 12228 was determined in vitro. Dried powder of flower, leave and stem parts of this plant were kept in acetone and methanol for 3 days in dark place. Disc diffusion method was used to determine the antibacterial activity of *Hypericum calycinum* L. Methanol-impregnated discs and acetone-impregnated discs were used as negative control. Also commercial antibiotic discs (Clindamycin (10 µg), Tetracycline (10 µg) and Amoxicillin-Clavulanic acid (30 µg)) were utilized as positive control. According to the results of the present study, the crude extracts of *Hypericum calycinum* L. prepared in acetone and methanol have been found to have antibacterial activity against the test microorganisms. Extracts of *Hypericum calycinum* L. flower showed the largest inhibition zone (13 mm in diameter) against *S. mutans.* The antibacterial activities of methanol and acetone extracts of *Hypericum calycinum* L.(at the doses of 3200 µg/10µl, 1600 µg/10µl, 800 µg/10µl and 400 µg/10µl) against *Streptococcus salivarius*, *Streptococcus mutans*, *Staphylococcus epidermidis* and *Streptococcus mitis* have been reported in this study for the first time.

Keywords: Antibacterial activity, Disc diffusion method, *Hypericum calycinum* L.

INTRODUCTION

Development of drug resistance against the antibiotics has necessitated new antimicrobial substances, chemotherapeutic agents and agrochemicals that combine antimicrobial efficacy with low toxicity and minor environmental impact. Natural or synthetic non-conventional antibiotics are classified as modifiers of antibiotic activity due to improvingof antibiotic activity or reversal of antibiotic resistance by them [1]. Bacterial biofilms are consisted of community of microorganisms and self produced matrix, growing on a biotic surface and resistant to many antimicrobial agents. Thus, potential use of natural products is one of the new strategies for prevention and treatment of infectious disease [2]. Usage of medicinal and aromatic plants as natural products has an increasing interest in pharmaceutical, food, biotechnology, agricultural and cosmetic industries all over the world [3].

During the last 20 years, plant antimicrobials have received a renewed interest [4]. Secondary metabolites have received scientific interest due to their interesting biological activities and, sometimes, for their desirable pharmacological profiles [5]. Secondary metabolites of *Hypericum* have a growing interest because of their wide range of biological activities [6]. The genus *Hypericum* L. has attracted scientific attention in recent years, owing to its bioactive compounds including the phenolics [7]. *Hypericum* plants are widely used in folk medicine to treat the gastric ailments, burns, swelling, inflammation, anxiety, bacterial and viral infections [8]. *Hypericum* L. exists widely in temperate regions of the world and is a large genus of herbaceous or shrubby plants. It has been shown that a number of species of this genus possess various biological activities [9]. The genus *Hypericum* L. includes 469 species which have been exist every continent in the world, except Antarctica. 36 taxonomic sections is delineated by specific combinations of morphological characteristics and biogeographic distribution ranges in monographic work on the genus. Bioactive secondary metabolites of members of the genus were determined as naphthodianthrones (e.g. hypericin and pseudohypericin), flavonol glycosides (e.g. isoquercitrin and hyperoside), biflavonoids (e.g. amentoflavone), phloroglucinol derivatives (e.g. hyperforin and adhyperforin) and xanthones [10].

The genus is known as "sari kantaron, kantaron, binbirdelikotu, mayasilotu" in Turkey. In Turkish traditional medicine *Hypericum* species have been used for the treatment of burns, wounds, hemorrhoids, diarrhea and ulcers. *H. calycinum* is utilized as anti-asthmatic and to abolish the spasm. The traditional uses of *Hypericum* species in Turkey and investigation on the *Hypericum* spp. of Turkey were summarized and tabulated in the literature. Quantitative determination, chemical compositions, antioxidant activity, traditional uses and antidepressant activity of *H. calycinum* were determined by various investigations [11]. The plant material, origin and main components of the essential oils of *Hypericum* species have been summarized in literature. Main components of *H. calycinum* L. (aerial parts) essential oil from Turkey are α-Pinene (24.1%) and β-pinene (14.2%) [12, 13].

The aim of this study was to determine the antibacterial activity of crude extract of aerial parts of *Hypericum calycinum* L. against *Streptococcus mitis*, *Streptococcus salivarius*, *Streptococcus mutans* and *Staphylococcus epidermidis.*

MATERIALS and METHODS

Materials

Hypericum calycinum L.(flowering aerial parts) were collected from natural populations (Province-at an altitude of 1200 m in the Güneyköy village, Yalova, Turkey) in May 2014 in the flowering phase. The species was identified by Dr. Mehmet Sağıroğlu and Serap Dalgıç, Department of Biology, Faculty of Science and Arts, Sakarya University, Sakarya, Turkey. Test microorganisms used in present study were *Streptococcus mitis* CNCTC 4/77, *Streptococcus salivarius* CNCTC 64/59, *Streptococcus mutans* CNCTC 8/77 and *Staphylococcus epidermidis* ATCC 12228 which were supplied from Microorganism Culture Collections

Research and Application Center of Istanbul University.

Preparation of Extracts

Flower, leave and stem parts of *Hypericum calycinum* L. were dried in shade for 7 days. Dried flowers, leaves and stems of this plant were grinded by using mill. Plant powders were kept in chemical solvents for three days at a rate of 1:10 (w/v). Acetone (Merck) and methanol (Merck) were used as chemical solvents. The solvents in the obtained extracts were evaporated by using rotary evaporator (Heidolph Laborota 4000 efficient) under vacuum at 50°C for 15 minutes. The extract concentrations were adjusted by adding own solvent to each extract at the doses of 3200 µg/10µl, 1600 µg/10µl, 800 µg/10µl and 400 µg/10µl.

Determination of Antibacterial Activity

Test microorganisms were inoculated to Tryptic Soy Broth (Merck) and were incubated at 37°C for 24 hours to obtain overnight bacterial cultures. Bacterial suspension was prepared from overnight culture and was adjusted to 0.5 McFarland by using a densitometer (Biosan). Disc diffusion method was used to determine the antibacterial activity of leaves, flowers and stems of *Hypericum calycinum* L. Sterile discs (6 mm in diameter, Rotilabo) were impregnated with the 10 µl of prepared extracts and were allowed to dry. Methanol-impregnated discs and acetone-impregnated discs were used as negative control. Also commercial antibiotic discs (Clindamycin (10 µg), Tetracycline (10 µg) and Amoxicillin-Clavulanic acid (30 µg)) were utilized as positive control. 0.5 McFarland bacterial suspension was inoculated to Mueller Hinton Agar by using sterile swabs. The discs impregnated with extracts were slightly pressed onto the Agar. Agars which were prepared by using this protocol were incubated at 37°C for 24 hours. The diameters of the inhibition zone (IZs) were measured by using electronic digital caliper. Experimental studies were performed two times under aseptic conditions and the diameters of IZs were the average of two replicates.

RESULTS

Present study analyzed the antibacterial activity of leave, stem and flower parts of *Hypericum calycinum* L. Table 1 shows the diameter of the inhibition zone against the test microorganisms. Test results showed that the greatest inhibitory effect was determined against *S. mutans*. Flower of *Hypericum calycinum* L.extracts showed more antibacterial activity than the stem and the leave of this plant extracts against the test microorganisms.

Table 1. Inhibition zone diameters of *Hypericum calycinum* L.extracts

Extract (µg/disc)		Inhibition zone diameters (mm)			
		S. mitis	*S. mutans*	*S. salivarius*	*S. epidermidis*
FLOWER	Acetone				
	3200	9.8	11.4	nt	10.2
	1600	9.6	11.1	11.5	9.8
	800	9.6	10.7	10.2	9.6
	400	9.6	10.3	9.9	9.2
	Methanol				
	3200	11.1	13.0	12.7	10.4
	1600	10.7	12.9	12.1	8.6
	800	9.1	11.6	11.7	8.1
	400	8.9	11.0	11.5	7.9
LEAVE	Acetone				
	3200	9.4	9.5	8.7	8.2
	1600	9.2	9.3	10.1	8.2
	800	8.9	9.1	10.2	8.2
	400	8.7	8.9	10.5	0
	Methanol				
	3200	9.1	9.2	10.4	9.1
	1600	9.0	8.8	9.7	8.9
	800	7.8	0	9.3	8.3
	400	7.6	0	0	0
STEM	Acetone				
	3200	10.2	9.4	11.0	0
	1600	9.5	9.7	10.4	0
	800	8.8	9.9	9.7	0
	400	8.0	9.4	8.9	0
	Methanol				
	3200	9.0	9.4	10.1	9.1
	1600	8.9	8.4	9.7	8.7
	800	7.3	0	8.8	8.1
	400	7.3	0	0	7.8
ANTIBIOTICS	Clindamycin (10 µg/disc)	34	48	40	35
	Tetracycline (10 µg/disc)	31	26	28	8
	Amoxicillin-Clavulanic acid (30 µg/disc)	42	5	45	32

DISCUSSION

To our knowledge, the antibacterial activity of methanol and acetone extracts of *Hypericum calycinum* L. against *Streptococcus salivarius, Streptococcus mutans, Staphylococcus epidermidis* and *Streptococcus mitis* has been reported in present study for the first time.

Green plants have been used to treat various diseases for many centuries. Gottshall *et al.* reported that ethanol extract of *Hypericum calycinum* leaves have shown antibacterial activity against *M. tuberculosis* [14]. The antimicrobial activity of flower and leave of *H. calycinum* against eight microorganisms (*Candida utilis, Micrococcus luteus, Staphylococcus aureus, Bacillus subtilis, Mycobacterium smegmatis, Escherichia coli, Salmonella typhimurium, Candida albicans*) were investigated by Sakar and Tamer. It was observed that flower of *H. calycinum* had more antimicrobial activity than leave [15].

Gibbons *et al.* pointed out that the inhibition zones of *Hypericum calycinum* chloroform and methanol extracts against methicillin-resistant *Staphylococcus aureus* (MRSA strain XU212) were 16 and 12 respectively. It has been determined that the minimum inhibitory concentration of chloroform extract of *Hypericum calycinum* against MRSA strain XU212 was 256 µg/ml [16]. Antimicrobial activity of *Hypericum calycinum* essential oil against *S. aureus, B. subtilis, E. faecalis, E. coli, C. albicans* was analyzed as minimum inhibitory concentration(MIC values in the range of 78-2500µg/ml) by Maggi *et al.* [17].

It has been observed that the ethanolic extract of *Hypericum calycinum* had antimicrobial activity (inhibition zone values in the range of 6-16 mm) against *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Bacillus cereus* ATCC 7064, *Micrococcus luteus* La 2971, *Proteus vulgaris* ATCC 8427, *Pseudomonas aeruginosa* ATCC 27853, *Debaryomyces hansenii* DSM 70238, *Kluyveromyces fragilis* ATCC 8608 and *Rhodotorula rubra* DSM 70403 [18]. Ethanol extract from *Hypericum calycinum* was screened by Nogueira *et al*. for its antimicrobial activity against *E. coli, S. aureus, S. faectum*, 4 non-tuberculous Mycobacterium species, a reference strain H37Rv and 4 drug-resistant strains of *Mycobacterium tuberculosis*, as well as 4 drug-resistant clinical isolates [19].

In this study we report that the crude extracts obtained from flowers, leaves and stems of *Hypericum calycinum* L. have antibacterial activity against *Streptococcus mitis* CNCTC 4/77, *Streptococcus salivarius* CNCTC 64/59, *Streptococcus mutans* CNCTC 8/77 and *Staphylococcus epidermidis* ATCC 12228. Results of the present study may be beneficial for further studies on this species to design new herbal drug after clinical studies.This plant may be used as natural antimicrobial in foods, nutraceuticals, cosmetic preservatives and pharmaceuticals after performing the required toxicological studies because of some potentially harmful compounds exist in the extracts or their fractions. We hope that the results of present study will provide development of new compounds with better activity than agents currently used.

Acknowledgements

The authors are very thankful to Dr. Mehmet Sağıroğlu and Serap Dalgıç for their helps.

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