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Original Article

Antimicrobial Properties of *Croccus sativus L.*

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Abstract

Antimicrobial activity of different parts of *Croccus sativus L.* (saffron) including stigma, stamen, leaves and colora, extracted by various solvents, were tested against different bacteria (*Microccucos luteus, Staphylococcus epidermitis, Staphylococcus aureus* and *E. coli*) and fungi (*Candida albicans, Aspergillus niger and Cladospourium sp*) by cup plate diffusion method. Minimal Inhibitory Concentration (MIC) values of each active extract were determined. The results obtained show strong activity of the ethyl acetate extract of various plant parts of the plant (except leaves) against bacteria and fungi used as test organisms.

Keywords: Croccus sativus; Saffron; Antimicrobial activity.

Introduction

Over the last few decades the great advances in our understanding of the causes of transmission, treatment and prevention of infectious diseases have fostered complacency about infections in a society which is well nourished and has access to vaccines, antibiotics and other drugs. However infections remain the leading causes of mortality worldwide (1). The increasing demand in finding novel antimicrobial agents, especially antifungal agents capable of overcoming deep-seated Mycoses, and resistance induction has diverted scientists attention toward natural products. Regarding the diversity in the population of plants around the world, in this series of projects we tried to focus mainly on the plants native to Iran.

Croccus sativus L.(saffron) (Fam.Iridaceae) is a widely used plant, especially as a food additive and coloring agent, in Iran. Due to the large production of this plant and only the use of its stigma, it is very important to find some other uses from the other parts of saffron. In this article the results obtained from studying the antimicrobial activity of different parts of *Croccus sativus L.*, have been reported.

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Experimental

Plant material

Different parts of *Croccus Sativus L.*, including stigma, stamen and colora were collected during November (1998) and leaves were collected during April from province of Khorasan in Iran. Next, the plant was identified and stored in the Herbarium of Pharmacognosy Department, School of Pharmacy, Shaheed Beheshti University of Medical Sciences (voucher number 99- 578).

Preparation of plant extracts

For the preparation of various extracts from different parts of *Croccus sativus* L. (stigma, stamen, leaves and colora), they were shade dried at room temperature and powdered by electric blender. 25g of each of the dried and powdered materials were macerated separately with 200 ml of ethyl acetate, ethanol and petrolium ether for 48h. Extracts were

Table 1. Antimicrobial activity of different parts of *Crocus sativus* extracted by various solvents (100mg/ml extract)

Extract	stigma 2g (w/w) a			stamen 2.4 g (w/w)			leaves 2.9g (w/w)			colora 1.9g (w/w)		
organisms	1b	2	3	1	2	3	1	2	3	1	2	3
S. aureus	-	-	-	-	18	-	-	-	12	14	13	-
S. epidermidis	-	12c	-	-	21	-	-	-	-	-	13	14
E.coli	-	-	-	-	16	-	-	-	-	-	-	-
M. luteus	-	-	-	-	21	-	14	-	-	-	9	-
C. albicans	-	19	-	-	-	-	-	-	-	-	9	-
Cladosporium Sp.	-	15	14	-	15	13	-	-	-	-	14	-
A. niger	-	16	-	-	17	-	-	-	-	-	-	-

a, g extract resulted from 25 gr powder of different parts of the plant

b, 1 = ethanolic extract, 2 = ethyl acetate extract and 3 = petrolium ether extract

c, = mm zone of inhibition

concentrated under reduced pressure. The condensed products were weighed and kept at 4 ºC prior to test.

Test microorganisms

Strains, including fungi and bacteria were obtained from Persian Type Culture Collection (PTCC).

Staphylococcus aureus PTCC 1112, *Staphylococcus epidermidis* PTCC1114, *Escherichia coli* PTCC 1037, *Micrococcus luteus* PTCC 1109, *Candida albicans* PTCC 5027, *Cladosporium* sp. PTCC 5202 and *Aspergilus niger* PTCC 5010 were used as test organisms.

Antimicrobial assay

Antimicrobial activity of the above mentioned extracts was determined, using a slightly modified cup plate method (2). Muller Hinton agar was used for the growth of bacterial strains and Malt extract agar was used for the growth of fungi. Each organism was separately suspended in a normal saline solution and transmittance (T) of 75 - 77% at 530 nm was made, which is equal to 10 6 CFU/ml. Plant extracts were dissolved in DMSO at a concentration of 100 mg/ml. Each plate was inoculated with 20µl of microbial suspension. 100µl of extract was added to each cup. The plates containing bacteria were incubated at 37 ºC for 24h and those containing fungi were incubated at 25 ºC for 48h. The positive antimicrobial activity was read based on growth inhibition zone and compared with the solvent, as control. In order to determine the relative minimum inhibitory concentration values, which are the minimum concentrations of agents showing growth inhibition zone when examined visually, extracts were dissolved in DMSO to make a concentration of 100 mg/ml. The extracts were then diluted in a two-fold manner to make different concentrations. 100µl of the active extracts were then added to each cup. All the tests were repeated in duplicates.

Results and Discussion

As shown (Table 1), the ethyl acetate extract of stigma, stamen and colora exhibit activity against the majority of the fungi and bacteria tested. No activity was observed when the ethyl acetate extract of leaves was used against test organisms at the concentration of 100 mg/ml. The relative antifungal activity of ethyl acetate of stigma was higher than stamen. In contrast, the relative antibacterial activity of ethyl acetate extract of stamen was higher than the other parts of *Croccus sativus*. Ethyl acetate extract of various parts of the plant were also tested for

Table 2. Relative minimum inhibitory concentration of ethyl acetate extract of different parts of Croccus sativus.

Organisms	Stigma	Stamen	Colora
S. aureus	-	25	50
S. epidermidis	50*	12.5	25
E. coli	-	50	-
M. luteus	-	12.5	50
C. albicans	6.25	-	50
Cladosporium sp.	12.5	50	25
A. niger	12.5	25	-

* mg/ml

their relative minimum inhibitory concentration.

The MIC values are shown in Table 2. The results reported here can be affected by factors such as the extraction method, yield of extract, antifungal and antimicrobial test methods, etc. The presence of activity within the extracts used in the preliminary tests may well depend on extracts concentration. For example, extract concentrations ranging from 100 µg/ml (3) up to 20 mg/ml have been used in each test (4).

The results obtained from this study indicate that the different parts of *Croccus sativus*, especially stamen and colora, can also be used as a good source of antimicrobial agents.

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