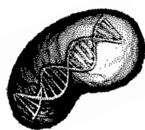

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REGULAR ARTICLE

ANTIMICROBIAL ACTIVITY OF *TUSSILAGO FARFARA* L.

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ABSTRACT

In this study, ethanolic extracts of *Tussilago farfara* L. which had been described in herbal books, were screened for their antimicrobial activity. The following strains of bacteria for antimicrobial activity were used *Escherichia coli* CCM 3988, *Serratia rubidea* CCM 4684, *Staphylococcus epidermis* CC 4418, *Lactobacillus rhamnosus* CCM 1828, *Pseudomonas aeroginosa* CCM 1960 and *Enterococcus raffinosus* CCM 4216. The yeast strain used in this study was *Saccharomyces cerevisiae* CCM 8191 using disc diffusion method and microbroth dilution technique. The highest antibacterial activity of *Tussilago farfara* L. ethanolic extract was measured in Grampositive bacteria *Lactobacillus rhamnosus* (6.67±1.53 mm) and lower in yeast *Saccharomyces cerevisiae* (1.67±0.58 mm) with disc diffusion method used. The ethanolic extract present an important activity against *Saccharomyces cerevisiae* (MIC50=24 μg.ml^{-1}; MIC90=25.69 μg.ml^{-1}) and *Serratia rubidaea* (MIC=48.01 μg.ml^{-1}; MIC90=51.26 μg.ml^{-1}) with microbroth dilution technique used.

Keywords: antimicrobial activity, pathogens, *Tussilago farfara* L., ethanolic extract

INTRODUCTION

The flower buds of *Tussilago farfara* L. (*Compositae*), commonly known as coltsfoot or "Kwandong Hwa" in China and Korea, have long been used as a traditional medicine for the treatment of bronchitis and asthma (**Chang-Tian *et al.,* 2012**). It is native to several locations in Europe and Asia. In China, it is mostly distributed in the northwestern and some of the southwestern areas.

Although some pure compounds such as a number of phenolics, mucopolysaccharides and water-soluble polysaccharides have been isolated from the flower buds of *T. farfara*, no flavonoids have been isolated. *T. farfara* has several known pharmacological activities, namely, antimicrobial activity (**Kokoska *et al.,* 2002**), inhibitory activity against nitric oxide synthase and antagonistic activity on platelet-activating factor receptor. Although, some of the biologic effects of these constituents have been clarified, an investigation of the pharmacological activities of the pure flavonoids in *T. farfara* seems to be interesting (**Kim *et al.,* 2006**).

The extracts of farfarae flos exhibit antioxidant effect, antimicrobial activity, and inhibitory effects on no synthesis in lps-activated macrophages and diacylglycerol acyltransferase activity (**Ryu *et al.,* 1999; Kokoska *et al.,* 2002; Cho *et al.,* 2005**). Recently, it has been reported that tussilagone (tsl), the major component of farfarae flos, suppresses inos and cox-2 expression in lps-stimulated bv-2 microglial cells (**Lim *et al.,* 2008**). However, the molecular mechanism by which tsl exerts antiinflammatory activity is not elucidated well.

Many efforts have been made to discover new antimicrobial compounds from various kinds of sources such as micro-organisms, animals, and plants. One of such resources is folk medicines **(Salamon and Fejer, 2011; Gruľová *et al*., 2012)**. Systematic screening of them may result in the discovery of novel effective compounds (**Tomoko *et al.,* 2002**). The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the spectre of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies (**Janovska *et al.,* 2003**).

In this study, ethanolic extract of *Tussilago farfara* L. which had been described in herbal books, were screened for their antimicrobial activity.

MATERIAL AND METHODS

Preparation of crude extracts

Flower and stem samples of *Tussilago farfara* L. were dried and the dried material was ground to a coarse powder. Fifty grams of the sample of dried plant material was extracted extensively in 400 ml ethanol for two weeks at room temperature with gentle shaking. The extract was filtered through filter paper (Whatman no. 54) under vacuum followed by drying by rotary evaporation (rotary evaporator Stuart RE300DB, England).

Tested microorganisms

The following strains of bacteria were used: *Escherichia coli* CCM 3988, *Serratia rubidaea* CCM 4684, *Staphylococcus epidermis* CC 4418, *Lactobacillus rhamnosus* CCM 1828, *Pseudomonas aeroginosa* CCM 1960 and *Enterococcus raffinosus* CCM 4216. The yeast strain used in this study was *Saccharomyces cerevisiae* CCM 8191.
The bacteria were grown overnight at 37 °C in Mueller-Hinton Broth (Oxoid, England) at pH 7.4 and yeast at 30°C in Sabouraud's glucose broth (Oxoid, England).

Antibacterial activity with disc diffusion method

Antimicrobial activity of each plant extract and was determined using a disc diffusion method. Briefly, 100 µl of the test bacteria were grown in 10 ml of fresh media until they reached a count of approximately 10^5 cells.ml^{-1}. One hundred microliters of the microbial suspension was spread onto nutrient agar plates. The extracts were tested using 6 mm sterilized filter paper discs. The diameters of the inhibition zones were measured in millimeters. All measurements were to the closest whole millimeters. Each antimicrobial assay was performed in at least triplicate. Filter discs impregnated with 10 µl of distilled water were used as a negative control.

Minimum inhibitory concentration MIC

Minimum inhibitory concentrations (MICs) determination was performed by a serial dilution technique, using 96-well microtitre plates. The bacterial inoculum applied contained

approximately 1.0 x 10^5 cells in a final volume of 100 µl.well^{-1}. The pure plant material tested were dissolved in DMSO (512 to 1 µg.ml^{-1}) and added to broth medium with bacterial and yeast inocula. The microplates were incubated for 16 – 20 hours at 37 °C. The lowest concentrations without visible growth determined as different between start concentration and final concentration of solution by ELISA Reader (Biotek ELx808iU) were defined as concentrations which completely inhibited bacterial and yeast growth (MICs). The first row on 96-well microtitrate plate was control of sterility and final row was control of growth without pure compound of plant material.

RESULTS AND DISCUSSION

For many years, medicine had depended exclusively on leaves, flowers and barks of plants; only recently have synthetic drugs come into use and in many instances, these are carbon copies of chemicals identified in plants (**Doughari and Manzara, 2008**).

The *in vitro* antibacterial activity of the *Tussilago farfara* L. ethanolic extract was tested by using disc diffusion method with the microorganisms as seen in table1. The highest antibacterial activity of *Tussilago farfara* L. ethanolic extract was measured in Grampositive bacteria *Lactobacillus rhamnosus* (6.67±1.53 mm) and lower in yeast *Saccharomyces cerevisiae* (1.67±0.58 mm).

Table 1 Antibacterial activity of *Tussilago farfara* L. against the test bacteria

Name of tested microorganisms	inhibition zones in mm
***Escherichia coli* CCM 3988**	3.33±1.53
***Serratia rubidaea* CCM 4684**	5.33±0.58
***Staphylococcus epidermis* CCM 4418**	4.67±2.08
***Lactobacillus rhamnosus* CCM 1828**	6.67±1.53
***Pseudomonas aeroginosa* CCM 1960**	4.67±2.08
***Enterococcus raffinosus* CCM 4216**	3.33±0.58
***Saccharomyces cerevisiae* CCM 8191**	1.67±0.58

In the study **Uzun et al. (2003)** twelve of the twenty four tested extracts have a totally or partial antimicrobial activity. The activities usually are seen with the petroleum ether extract, except the activity of *Trachystemon orientalis*. Each extract is made in double, to make sure that the activity is correct and not result of random occurrences. For each plant

species that shows activity the zone size is measured and these sizes are more or less the same. Except *Equisetum telmateia*, which has an activity against *Candida albicans*, all the other plant species inhibited bacteria, mainly *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Salmonella typhi*.

The greater susceptibility of Grampositive bacteria has been previously reported for South American (**Paz *et al.,* 1995**), African (**Kudi *et al.,* 1999; Vlietinck *et al.,* 1995**) and Australian (**Palombo and Semple, 2001**) plant extracts. Susceptibility differences between Grampositive and Gramnegative bacteria may be due to cell wall structural differences between these classes of bacteria. The Gramnegative bacterial cell wall outer membrane appears to act as a barrier to many substances including antibiotics (**Tortora *et al.,* 2009**).

In contrast to the **Palombo and Semple (2001)** report, many of the Australian native plant extracts examined in the present report also had significant activity towards Gramnegative bacteria. These differences relate to the different species studied, but may also relate to the extract concentrations tested. Some of the extracts tested in this study were tested at concentrations as high as 30-40 mg extracted material per ml.

Table 2 Minimum inhibitory concentration of *Tussilago farafara* L. ethanolic extract

Name of tested microorganisms	μg.ml^{-1}	
	MIC 50	**MIC 90**
***Escherichia coli* CCM 3988**	64.00	71.53
***Serratia rubidaea* CCM 4684**	48.01	51.26
***Staphylococcus epidermis* CCM 4418**	191.85	203.97
***Lactobacillus rhamnosus* CCM 1828**	64.00	71.53
***Pseudomonas aeroginosa* CCM 1960**	64.00	71.53
***Enterococcus raffinosus* CCM 4216**	64.00	71.53
***Saccharomyces cerevisiae* CCM 8191**	24.00	25.69

The determination of the MIC by means of the microbroth dilution method (Table 2) showed that plant extract tested exhibited an antimicrobial effect against some of the seven tested microorganisms. The results of the bioassays showed that extract exhibited moderate to appreciable antibacterial activities against all bacteria. However, this activity varies with the kind of bacteria. The ethanolic extract present an important activity against *Saccharomyces*

cerevisiae (MIC50=24 $\mu g.ml^{-1}$; MIC90=25.69 $\mu g.ml^{-1}$) and *Serratia rubidaea* (MIC=48.01 $\mu g.ml^{-1}$; MIC90=51.26 $\mu g.ml^{-1}$).

The results of **Janovská *et al.* (2003)** study showed that the extracts from *Sanguisorba officinalis*, *Tussilago farfara* (aerial part; rhizome), *Chelidonium majus* (root), *Tribulus terrestris* (aerial part) and *Schisandra chinensis* (leaves) possessed antimicrobial activity. Although the plants differ significantly in their activities against the microorganisms tested, more of the extracts showed antimicrobial activity against *B. cereus* and *S. aureus* than against *E. coli*, *P. aeruginosa* and *C. albicans*.

CONCLUSION

In conclusion, it is our firm belief that the study of medicinal plants as *Tussilago farfara* L. as antimicrobial agents is necessary for gaining insight into medicinal flora and their real value, but the use of a standard method for investigation is essential. Likewise, the concentrations or dilutions used must be appropriate. Moreover, research in this area should be carried on until the agent responsible for the activity has been determined or, as the case may be, the most active fraction or extracts have been discovered. Finally, different kinds of studies on the mechanisms of action, interactions with antibiotics or other medicinal plants or compounds, and the pharmacokinetic profile of the extracts should be given high priority.

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