INTRODUCTION

Medicinal plants are a source of basic raw materials for different industries such as pharmaceutical, cosmetic, perfumery, food, etc. The whole plant or its different parts may be of high value because of its therapeutic, medicinal, aromatic or savory qualities. They also play a vital role as an antimicrobial agent. There has been resurgence in the consumption and demand for medicinal plants because the resistance to antimicrobial agents such as antibiotics is emerging worldwide of variety of organisms and thus multiple drug resistant organisms pose serious threats to society. Hence, plant derived antimicrobial agents have received considerable attention in recent years (Sathiyah et al., 2008).

The pseudo-fruit of Rosa canina L. (Rosaceae) consists of a u-shaped receptacle with numerous achenes inside. The pseudo-fruit is rich in Vitamin C and their fresh and dried products are frequently used as an herbal tea (Montazeri et al., 2011; Kerényi-Nagy et al., 2013). Rose pseudo-fruit is traditionally apply for the prevention and therapy of common cold, prevention of inflammation of the gastric mucosa and gastric ulcer, and for gallstones and biliary complaints. It is also used as a laxative, for disorders of the kidney and the lower urinary tract. In addition, it is used as a diuretic in case of dropsy. Likewise, it is used as an astringent (Delormean Orhan et al., 2007; Wenzig et al., 2009; Fecka, 2009) and in the treatment of various inflammatory diseases as a source of Vitamin C (Chrubasik et al., 2006). Rose pseudo-fruit, which is marketed as a food supplement, has been shown to reduce osteoarthritis symptoms in clinical trials (Rein et al., 2004).

Some works have been published on chemical composition of some rose species fruits, especially on Rosa canina. It was previously reported that R. canina fruits, which contain high phenolic and flavonoid contents, have antioxidant, antimutagenic and anticarcinogenic effects. Passing the different biochemical assays, including the ability to reduce the hydroxyl radical south (OH•), hydrogen peroxide (H2O2), Trolox equivalent antioxidant capacity, metal ion chelating and free radical scavenging activity, gives to fruits of R. canina the natural antioxidant properties and could replace the synthetic additives (Kilicgun and Altiner, 2010; Egea et al., 2010). The antioxidant activity of leaf extracts of R. canina were also reported after treatment with ABTS test, 2,2’-azinobis (3- ethylbenzthiazoline-6-acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) methods (Ghazghazi et al., 2008). Additionally the recent studies revealed that R. canina extracts were effective for the inhibition of growth and biofilm formation in methicillin-resistant Staphylococcus aureus (MRSA) (Serteser et al., 2008; Quave et al., 2008).

METHODOLOGY

MATERIAL AND METHODS

The flowers of R. canina, which is growing in wild in Slovakia, were collected in May 2013 from Nitra-Zobor, Vrhové-Barani dvor, Rišňovce, Modra pažíť places in Slovakia.
Preparation of plant extracts

After drying, flowers of *Rosa canina* were crushed and 10 g of material was soaked separately in 100 mL of ethanol p.a. (96%, Sigma, Germany) during two weeks at room temperature in the dark. Exposure to sunlight was avoided to prevent the degradation of active components. Then the ethanol plant extracts were subjected to evaporation under reduced pressure at 40 °C in order to remove the ethanol (Stuart RE300DDB rotary evaporator, Bibby scientific limited, UK, and vacuum pump KNF N838.1.2KT.45.18, KNF, Germany). For the antimicrobial assay, the drying flowers extracts were dissolved in ethanol and methanol, which were used as the solvents.

Test microorganisms

Five strains of microorganisms were tested in this research. Two Gram-negative bacteria include *Escherichia coli* CCM 3988 and *Pseudomonas aeruginosa* CCM 1960, three microscopic filamentous fungi strain *Aspergillus niger*, *Fusarium culmorum* and *Alternaria alternata*. Bacterial strains were collected from the Czech Collection of Microorganisms (Masaryk University, Czech Republic) and microscopic filamentous fungi were collected from the Department of Microbiology, Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture in Nitra, Slovakia. The bacterial isolates were cultivated in Muller Hinton broth (Imuna, Slovakia) at 37 °C for 24 hours and microscopic filamentous fungi culture was cultivated in Malt extract broth (Biomark, India) at 30 °C for 24 hours.

Antimicrobial activity with disc diffusion method

Antimicrobial activity of flowers extract was determined using a disc diffusion method. Briefly, a 100 µl of the test bacteria and microscopic fungi were grown in 10 ml of fresh media until they reached a count of approximately 10^7 cells/ml. An amount of 100 µl of the microbial suspension was spread onto Mueller Hinton agar and Malt agar plates (Biomark, India). The extracts were tested using 9 mm sterilized filter paper discs impregnated with the tested suspension. The diameters of the inhibition zones were measured in millimeters. All measurements were to the closest whole millimeter. Each antimicrobial assay was performed at least triplicate. Filter discs impregnated with a 10 µl of distilled water were used as a negative control.

RESULTS AND DISCUSSION

In our study the best antimicrobial effect of *Rosa canina* flower ethanolic extract was found against Gram-negative bacteria – *Pseudomonas aeruginosa* and *Escherichia coli* than against microscopic fungi – *Aspergillus niger*, *Fusarium culmorum* and *Alternaria alternata* (Figure 1).

Extract from *Rosa canina* L. petals (rose red) strikingly increases the effectiveness of several antibiotics against methicillin-resistant *Staphylococcus aureus* (Rossnagel and Willich, 2001). Trovato et al., 2000 study has demonstrated strong activity of *Rosa canina* extract against *Candida albicans* strains isolated from clinical samples obtained in case of acute vaginitis. Additionally, the effect of anthocyanin preparation isolated from the flower petals of *Rosa canina* was studied in Chinese hamster fibroblasts and Vicia faba seedlings in respect to cytogenetic damage and mouse survival and there were pronounced radioprotective effects demonstrated without any toxic consequences (Trovato et al, 2000).

Figure 1: Antibacterial activity of *Rosa canina* flower ethanolic extract against microorganisms (in mm)

Result data of Hırılkar and Agrawal, 2010 showed that low dilution of alcoholic extract exhibited higher antimicrobial activity comparing with higher dilution. Petroleum and its extract showed maximum of 29mm of inhibition zone for *Pseudomonas aeruginosa* in comparison to other bacterial strains. Alcoholic extract showed higher inhibitory effect on *Streptococcus pneumoniae* (30mm of inhibition zone), *Enterobacter aerogens* (28mm), *Staphylococcus epidermidis* (25mm), *Bacillus subtilis* (30mm), *Pseudomonas aeruginosa* (32mm). Aqueous extract showed higher inhibition against *E.coli* (21mm), *Enterobacter aerogens* (25mm) and *Bacillus subtilis* (28mm) in comparison to other bacterial strains. The zone of inhibition was higher to antibiotic at the highest concentration in the case of *Streptococcus pneumoniae* and *Pseudomonas aeruginosa* then the antimicrobial activity of *Rosa* petals with control antibiotic compared. Study showed that the average relative antimicrobial activity was found the highest for alcoholic extract (25mm), followed by aqueous (19mm) and petroleum (18mm).

Darokar et al. 1998 was found Gram-positive *Bacillus subtilis* strain ATCC 6015 and Gram-negative bacterium *Pseudomonas aeruginosa* ATCC 25668 strain to be the most sensitive to rose petals; and the petals of 18 varieties were active against ATCC 6015 and those of 13 varieties against ATCC 25668. Among the Gram-positive bacteria, *Streptococcus mutans* and *Mycobacterium smegmatis* were relatively more tolerant to rose petals compared to *Staphylococcus epidermidis*, *S. aureus* and *B. subtilis*. Among the Gram-negative bacteria, the strains of *Escherichia coli*, *Enterococcus faecalis* and *Enterobacter aerogenes* were more tolerant to rose petals compared to *Klebsiella pneumoniae*, *Salmonella typhimurium* and *P. aeruginosa* strains (Darokar et al., 1998).

In our study the best antimicrobial effect of methanolic extract of *Rosa canina* flower was found against Gram-negative microorganisms and especially against *Escherichia coli* (Figure 2).

Figure 2: Antibacterial activity of *Rosa canina* flower methanol extract against microorganisms (in mm)

Ozturk Yilmaz and Erçisli, 2011 study clearly indicated that the methanolic extract of different *Rosa* taxa fruits has antibacterial activity against a number of bacteria and antibacterial activity was differed among taxa tested against bacteria. Regarding the taxa tested, *Rosa pisiiformis* extract inhibited the growth of *Yersinia enterocolitica*, *Streptococcus aureus*, *Bacillus cereus* and *Salmonella typhimurium* with 0-13 mm inhibition zone. *Rosa canina* extract inhibited the growth of *Yersinia enterocolitica*, *Enterococcus faecalis* and *Bacillus cereus* with 9-11 mm inhibition zone. *Rosa villosa* extract inhibited the growth of *Enterococcus faecalis* and *Bacillus cereus* with 10-12 mm inhibition zone. *Rosa damalis* subsp. *antalyensis* extract inhibited the growth of *Yersinia enterocolitica*, *Bacillus cereus* and *Klebsiella pneumoniae* with 10-14 mm inhibition zone. The *Rosa canina* fruit methanol extract atMIC = 2.0 mg/mL was the most effective against *S. aureus* and *C. albicans*. Also, with the exception of *B. cereus*, the water extract of *Rosa canina* demonstrated the antimicrobial activity against both Gram-positive and Gram-negative bacteria tested in this study, and the highest MIC was 3.5 mg/mL. The antibacterial activity of the *Rosa canina* acetone extract was detected against *E. coli*, *S. aureus* and *C. albicans*. Furthermore, the chloroform and n-hexan extracts, even in the concentration of 10 mg/mL, had no inhibition activity against all tested microorganisms (Montazeri et al., 2011).

The results obtained in Mishra et al. 2011 study showed that the leaves, stem and flower of *Rosa indica* (red) have bactericidal effect on pathogenic microorganisms. The methanolic extract showed better and improved antibacterial activity in comparison to ethyl acetate. The widest zone of inhibition (18mm) was determined by the methanolic extract of *Rosa indica* petals, followed by leaves (14mm) and stem (17mm) extracts. A remarkable 13mm zone of inhibition was recorded by ethyl acetate extract of *Rosa indica* stem against *P. aeruginosa*, while *E. coli* and *S. aureus* found insensitive. Similar type of investigations was reported earlier with ethanolic extracts (Sathiya et al., 2013).

CONCLUSION

The best antimicrobial effect of *Rosa canina* flower ethanolic extract was found against *Pseudomonas aeruginosa* and the best antimicrobial effect of *Rosa canina* flowers methanolic extract was found against *Escherichia coli*. Herbal medicines are valuable and readily available resource for primary health care and complementary health care systems. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value that have yet to be discovered, though large numbers of plants are constantly being screened for their antimicrobial effects. These plants may prove to be a rich source of...
compounds with possible antimicrobial activity, but more pharmacological investigations are necessary.

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REFERENCES


