Microbiological Communication

Biosci. Biotech. Res. Comm. 9(3): 517-522 (2016)

BBRC Bioscience Biotechnology Research Communications

Phytochemical analysis and antibacterial effects of *Calendula officinalis* essential oil

Shapour H. Chaleshtori, Mehrdad A. Kachoie* and Abdollah G. Pirbalouti

Herbal Medicine Department College of Food and Drug, Shahrekord Branch, Islamic Azad University, Shareakord Iran

ABSTRACT

Occurrence of antibiotic resistance in pathogenic strains of bacteria caused researchers to search for substitution of chemical antibiotics with natural products derived from plants. High levels of antibacterial and anti-oxidant materials make *Calendula officinalis* good for synthesis of antibacterial drugs. The present investigation was carried out to study the hemical components and antibacterial effects of the *C. officinalis* essential oil. Flowers of the *C. officinalis* were collected and transferred to the laboratory. Essential oil was extracted and the gas chromatography was applied to study the chemical components. Antibacterial effects of *C. officinalis* was studied using the disk diffusion method. 1,8-cineole (30.456%), γ-Terpinene (25.547%), Terpinolene (4.584%), α-Terpineol (4.490%) and Trans-α-ocinene (4.153%) were the most commonly detected components in the essential oil of the C. officinalis. Percent of chemical components had significant differences ($P < 0.05$). C. officinalis harbored the highest antibiotic effects on the Gram-negative bacteria ($P < 0.05$). The highest zone of inhibition was seen for the *E. coli* (13.31±1.24 mm) and *P. aeruginosa* (10.22±0.83 mm). The lowest zone of growth inhibition was seen for the *S. aureus* (3.14±0.27 mm). Statistically significant differences were seen between the types of bacteria and antibiotic effects of *C. officinalis* essential oil ($P < 0.05$). Careful prescription of antibiotics can control the occurrence of antibiotic resistance in pathogenic bacteria. We recommended use of *C. officinalis* essential oil as an anti-*E. coli* and *P. aeruginosa* agent.

KEY WORDS: *CALENDULA OFFICINALIS*, ESSENTIAL OIL, CHEMICAL COMPONENTS, ANTIBACTERIAL EFFECTS, PATHOGENIC BACTERIA

ARTICLE INFORMATION:

**Corresponding Author: Mehrdad.ataie@gmail.com*
Received 7th Sep, 2016
Accepted after revision 30th Sep, 2016
BBRC Print ISSN: 0974-6455
Online ISSN: 2321-4007
Thomson Reuters ISI ESC and Crossref Indexed Journal
NAAS Journal Score 2015: 3.48 Cosmos IF : 4.006

Online Contents Available at: http//www.bbrc.in/

INTRODUCTION

In despite of the high development of medical sciences, treatment of infectious diseases caused by pathogenic agents like bacteria, fungi and viruses is in trouble. These problems are mainly occurring due to the occurrence of antibiotic resistances (Dehkordi *et al*., 2012, 2014). Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. It is an increasingly serious threat to global public health that requires action across all government sectors and society (Momtaz *et al*., 2013; Dormanesh *et al*., 2014).

Resistant microorganisms are able to withstand attack by antimicrobial drugs, such as antibacterial drugs (e.g. antibiotics), antifungals, antivirals, and antimalarials, so that standard treatments become ineffective and infections persist, increasing the risk of spread to others (Davies and Davies 2010). Occurrence of these antimicrobial resistances caused chemical and pharmacological factories to use from novel sources for antibiotic producing. Application of medicinal plants for producing of antimicrobial agents had an ancient history.

Medicinal plants are a suitable sources of antimicrobial agents. *Calendula officinalis* (*C. officinalis*) is one of the most commonly used medicinal plants among Iranian people which is native to the Mediterranean regions (Pan *et al*., 2013). *C. officinalis*, commonly known as pot marigold, is an annual herb and belongs to Asteraceae family. Flowers are monoecious (individual flowers are either male or female, but both sexes can be found on the same plant) and are pollinated by Bees. It is noted for attracting wildlife. *C. officinalis* can be broadly applied as an antiseptic, anti-inflammatory and cicatrizing as well as a light antibacterial and antiviral agent (Pan *et al*., 2013; Arora et al., 2013; Efstratiou *et al*., 2012; Butnariu *et al*., 2012; Martins *et al*., 2014). The plant contains esquiterpenes glycosides, saponins, xanthophylls, triol triterpenes, flavonoids, volatiles, δ-cadinene, α-cadinol, 1,3,5-cadinatriene and α-muurolol which show anti-oxidative and antimicrobial effects (Pan *et al*., 2013; Arora *et al*., 2013; Efstratiou *et al*., 2012; Butnariu *et al*., 2012; Martins *et al*., 2014).

C. officinalis is used as anti-bacterial, analgesic, anthelmintic, anti-fungal, cholagogue, anti-spasmodic, anti-pyretic, hemostatic, antiseptic, anti-emetic, candidicide, anti-viral, astringent, bitter, anti-inflammatory, lymphatic, cardiotonic, carminative, diaphoretic, dermagenic, diuretic, immunostimulant, and uterotonic agent (Pan *et al*., 2013; Arora *et al*., 2013; Efstratiou *et al*., 2012; Butnariu *et al*., 2012; Martins *et al*., 2014). According to the high prevalence of pathogenic bacteria in Iranian cases of hospital infections, economic, cosmetic, and pharmaceutical values of *C. officinalis* and lack of published data on the identification of chemical components and antimicrobial activities of *C. officinalis*, the present study was carried out to evaluate the chemical components and antimicrobial effects of *C. officinalis* on standard strains of *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella typhi*, *Bacillus cereus* and *Staphylococcus aureus*.

MATERIALS AND METHODS

The present study was accepted by the ethical committees of the Islamic Azad University of Shahrekord, Iran. Written consent was signed by the Research Adjutancy of the Islamic Azad University of Shahrekord (IAUSHK 202542). Permission of this work was also taken from the Head of the Islamic Azad University of Shahrekord.

C. officinalis flowers were collected from the plains and mountains of the Zagros zone, Chaharmahl Va Bakhtiary province, Iran. Genus of collected flowers were identified and confirmed by the professor of the Medicinal Plants Research Center of the Islamic Azad University of Shahrekord, Iran. All flowers were collected on the February of 2015. Five-hundred grams of fresh flowers were hydro distilled separately for 3 h in an all-glass Clevenger apparatus in accordance with the British pharmacopoeia method (British Pharmacopoeia 1980).

In order to study the chemical compositions of *C. officinalis* flowers, the GC-mass analysis method was used using an Agilent 6890 Series II gas chromatograph (Palo Alto, USA) coupled to an Agilent 5973 quadrupole mass spectrometer with electron ionization mode (EI) generated at 70 eV (ion source at 230 °C and transfer line at 280 °C). The GC was performed using a J&W DB-5 (5% diphenyl- 95% dimethyl silicone) capillary column (30 m x 0.25 mm i.d. x 0.25 µm film), and helium was used as a carrier gas (1 mL min-1). The initial temperature was programmed from 35 °C to 60 °C (at 1 °C min-1), to 170 °C (3 °C min-1), to 200 °C (8 °C min-1), and to 280 °C (15 °C min-1), and maintained at 280 °C for 5 min. The injector port (splitless mode, 0.5 min) was at 250 °C. Retention indexes were calculated with reference to nalkanes. All compounds were identified by comparison of both the mass spectra (Wiley 275 library) and the retention index data found in the literature (Adams 1995).

The bacterial cultures were purchased from the Pasteur Institute of Iran. They were subculture onto Petri plate containing nutrient agar media (Merck, Germany). The strain of bacteria selected to assess susceptibility pattern against *C. officinalis* extract were *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 8739), *Salmonella typhi* (ATCC 14028), *Bacillus cereus* (ATCC 10987) and *Staphylococcus aureus* (ATCC 6538). Each

of the microorganisms was reactivated prior to susceptibility testing by transferring them into a separate test tubes containing broth and incubated overnight at 37°C at shaker.

Agar disc diffusion method was used for screening of antibacterial activity of *C. officinalis* extract (Efstratiou *et al.*, 2012). Bacterial strains were spread on to Nutrient Agar (NA, Merck, Germany) medium. Paper discs were separately impregnated with 25µl of the 0.5 mg/mL plant essential oil and placed on the inoculated agar plates. All the plates were allowed to stay at room temperature for 30 min to allow diffusion of the essential oil then incubated at 37 °C for 24 hrs. Interpreting of the diameter of the zone of inhibition was done according to the protocol of the Clinical Laboratory Standard Institute (CLSI 2012).

Antimicrobial effects of the *C. officinalis* essential oil were tested 3 times. Results were transferred to a Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) for analysis. Statistical analysis was performed using SPSS/20.0 software (SPSS Inc., Chicago, IL) for significant relationship between antimicrobial effects of *C. officinalis* essential oil on tested bacteria. The chi-square test and Fisher's exact 2-tailed test analysis were performed in this study. Statistical significance was regarded at a P value < 0.05.

RESULTS AND DISCUSSION

Frequency of chemical components in *C. officinalis* essential oil is shown in table 1. Totally, 40 different chemical components were detected in the essential oil of the *C. officinalis* essential oil. Totally, 1,8-cineole (30.456%), γ-Terpinene (25.547%), Terpinolene (4.584%), α-Terpineol (4.490%) and Trans-β-ocinene (4.153%) were the most commonly detected components in the essential oil of the *C. officinalis*. Significant statistical differences were seen between the frequency of chemical components (P < 0.05).

Table 2 represents the antibiotic susceptibility pattern of bacterial strains against essential oil of the *C. officinalis*. We found that the *C. officinalis* essential oil harbored the highest levels of antibiotic effects on the Gram-negative than Gram-positive bacteria (P < 0.05). The highest amount of diameter of the inhibition zone was seen for the E. coli (13.31±1.24 mm), followed by *P. aeruginosa* (10.22±0.83 mm). The lowest amount of diameter of the inhibition zone was seen for the *S. aureus* (3.14±0.27 mm). Statistically significant differences were seen between the types of bacteria and antibiotic effects of *C. officinalis* essential oil (P < 0.05).

As far as we know, the present investigation is the first prevalence report of chemical composition and antimicrobial effects of the *C. officinalis* on the pathogenic bacterial strains in Iran. We found that the essential oil of the *C. officinalis* had low antibacterial effects

Table 1: Frequency of chemical composition of *Calendula officinalis* essential oil.

Number	Chemical components	Frequency of components (%)
1	α-Thujene	0.459
2	α-Pinene	3.032
3	Camphene	0.256
4	Sabinene	0.293
5	P-Pinene	0.490
6	1-Octen-3-ol	0.291
7	1,8-cineole	30.456
8	p-cymene	2.495
9	α-Terpinene	0.283
10	β-caryophyllene	1.289
11	Trans-β-ocinene	4.153
12	Benzene acetaldehyde	1.354
13	γ-Terpinene	25.547
14	cis-Sabinene hydrate	2.928
15	Terpinolene	4.584
16	α-phellandrene	0.390
17	α-terpineol	0.676
18	Carvacrol	3.146
19	Terpinene-4-ol	0.369
20	α-Terpineol	4.490
21	n-Dodecane	0.279
22	Carvacrol methy ether	0.301
23	α-copaene	0.318
24	α-bourbonene	0.510
25	α-Terpinyl acetate	0.520
26	Eugenol	0.676
27	n-Tetradecane	3.146
28	(E)-Caryophyllene	0.369
29	α-muurolene	2.805
30	β-Bisabolene	0.622
31	(E)-γ-Bisabolene	0.301
32	Spathulenol	0.318
33	β-Eudesmol	0.510
34	α-Cadinol	0.520
35	γ-Cadinene	3.319
36	Cadina 1,4-diene	0.950
37	α-cadinene	1.434
38	α-Bisabolol	0.024
39	α-cadinol	0.616
40	T-muurolol	0.324

Table 2: Antibiotic susceptibility pattern of bacterial strains against *Calendula officinalis* essential oil.

Bacteria	Mean zone of inhibition (mm)
Pseudomonas aeruginosa	10.22±0.83a*
Escherichia coli	13.31±1.24a
Salmonella typhi	7.34 ±0.62b
Bacillus cereus	4.10±0.30c
Staphylococcus aureus	3.14±0.27c

*Dissimilar leathers in this column shows significant differences about P < 0.05.

on tested bacteria. Unauthorized and indiscriminate prescription of antibiotics are the main reasons for the high prevalence of resistance (low zone of inhibition) in the bacterial strains of our study.

S. aureus strains had the highest levels of resistance against *C. officinalis* essential oil. *S. aureus* strains of various previously published works harbored the highest levels of resistance against strong antibiotic agents such as penicillin, tetracycline, gentamycin, ampicillin, cefexime and ciprofloxacin which was similar to our results on the *C. officinalis* (Tokajian *et al.*, 2011; Virdis *et al.*, 2010; Udo *et al.,* 2008; Rijal *et al.*, 2008; Deng *et al.*, 2013). All of these researches have recommended synthesis, formulation and application of novel antimicrobial agents to overcome occurrence of high antibiotic resistance in the *S. aureus* strain of human and even animal clinical samples, but we found that the *C. officinalis* essential oil is not appropriate approach for synthesis of anti-*S. aureus* antibiotic.

In despite of the *S. aureus* and *B. cereus* which had the low diameter inhibition zone, *C. officinalis* essential oil had a high antibacterial effects on *E. coli* and *P. aeruginosa*. Probably, chemical components of this plant make it effective on the Gram-negative bacteria. Similar results have been reported previously.

In a study which was conducted by Efstratiou *et al.* (2012) (Efstratiou *et al.*, 2012), results showed that the *C. officinalis* extracts represented exceptional antibacterial activity against *P. aeruginosa, E. coli, K. aerogenes, E. faecalis* and *K. pneumonia* which was similar to our findings. Chakraborthy (2008) (Chakraborthy 2008) reported that the lowest Minimum Inhibitory Concentration (MIC) values of *C. officinalis* were observed for ethanol extract, chloroform extract, water extract and petroleumether extract against the bacteria. They showed that the extracts of *C. officinalis* leaves were significantly effective against both Gram-positive and especially Gram-negative organisms. High antimicrobial effects of the *C. officinalis* is due to its antimicrobial chemical components. Recent study revealed that triterpenoid like calendulaglycoside, triterpenoid saponin like faradiol, asorhamnetin3-O-neohesperidoside, quercetin and isorhamnetin are the main chemical components of the *C. officinalis* which are responsible for antioxidative, anti-cancer, antimicrobial, anti-inflammatory and wound healing effects (Muley et al., 2009).

We found that, 8-cineole (30.456%), γ-Terpinene (25.547%), Terpinolene (4.584%), α-Terpineol (4.490%) and Trans-β-ocinene (4.153%) chemical components had a high quantity in the *C. officinalis* essential oil. These components are anti-oxidant and antimicrobial materials of the *C. officinalis*. Results of the documented reports revealed that the main compounds within Calendula are the triterpenoids (Arora *et al.*, 2013; Butnariu *et al.*, 2012) which are claimed to be the most important anti-inflammatory and antimicrobial components within the plant. Other constituents identified in Calendula such as the saponins, micronutrients, flavonoids, and polysaccharides, may also be responsible for the antimicrobial, anti-inflammatory, antioxidant, and wound healing effect of the plant (Arora *et al.*, 2013; Butnariu *et al.*, 2012; Faria *et al.*, 2011).

The antimicrobial activity of essential oil of *C. officinalis* is attributed to its main chemical components including citral (aldehyde), geraniol (primary alcohol), eugenol (phenol), menthol (secondary alcohol) and cinnamic aldehyde (aldehyde) (Hartman and Coetzee 2002). Compounds such as linalool, citral, geraniol, or thymol are more antiseptic agents in the essential oil of the *C. officinalis* (Bruneton 2001). Butnariu and Coradini (2012) (Butnariu and Coradini 2012) reported that marigold is renowned for its antibacterial, anti-oxidant, cholagogic, diaphoretic and vulnerary properties. They showed that marigold extract is full of phenolic and saponin components which warranty its antibacterial and anti-oxidative activities. Rigane *et al.* (2013) (Rigane *et al.*, 2013) showed that Rutin, quercetin-3-O-glucoside, scopoletin-7-O-glucoside, isorhamnetin-3-O-glucoside and gallic acid were the most commonly flavonoid-based chemical components. They reported that *C. officinalis* (leaf extract) exhibited a better MIQ against *E. coli* and *S. aureus* than aqueous-methanolic flower extract having strong activity against *S. typhimurium* at lower quantities. The phenolic compounds and flavonoids found in *C. officinalis* could be responsible for its antimicrobial activity against *E. coli*, *S. typhimurium*, *S. aureus*, *C. albicans* and *A. niger*. Their results are in agreement with our findings.

CONCLUSION

In conclusion, we identified a large numbers of chemical components in the essential oil extracted from *C.*

officinalis. Totally, flavonoids and phenols are the main chemical components of *C. officinalis*. Good antibacterial effects of the *C. officinalis* especially on *E. coli* and *P. aeruginosa* but it was not effective on the *S. aureus* and *B. cereus*. Therefore, we recommended production of anti-*E. coli* and *P. aeruginosa* agent for treatment of the diseases caused by these two bacterium such as urinary tract infections, food-poisoning and burn and wound infections. Judicious prescription of antibiotics can control and eliminate the occurrence of antibiotic resistance in pathogenic bacteria.

ACKNOWLEDGEMENTS

The authors would like to thank Dr. F. Safarpoor Dehkordi of the Department of Food Hygiene and Quality Control, University of Tehran, Iran and all the staff members of the Medicinal and Aromatic Plants Research Center of the Islamic Azad University of Shahrekord, Iran for their important technical and clinical support. The present study was supported by the Islamic Azad University of Shahrekord, Iran (IAUSHK 1991394).

REFERENCES

Adams RP (1995). Identification of essential oil components by gas chromatography / mass spectroscopy. Illinois: Allured Publishing Corporation: 469.

Arora D, A Rani and A Sharma (2013). A review on phytochemistry and ethnopharmacological aspects of genus Calendula. Pharmacogn Rev. 7:179-87.

British Pharmacopoeia. (1980). H. M. S. Office. 2, London: 109-110.

Bruneton J. (2001). Farmacognosia. Zaragoza: 477.

Butnariu M and CZ Coradini (2012). Evaluation of Biologically Active Compounds from *Calendula officinalis* Flowers using Spectrophotometry. Chem Cent J. 6:35.

Chakraborthy GS. (2008). Antimicrobial activity of the leaf extracts of *Calendula officinalis* (linn.). J Herb Med Toxicol. 2:65-66.

Clinical and Laboratory Standards Institute (CLSI). (2012). Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement M100-S21. Wayne Pa.

Davies J and D Davies. (2010). Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev. 74:417-33.

Dehkordi FS, F Yazdani, J Mozafari and Y Valizadeh. (2014). Virulence factors, serogroups and antimicrobial resistance properties of *Escherichia coli* strains in fermented dairy products. BMC Res Notes. 7:217.

Deng JJ, JN Zhu, CL Yang, M Shu, GG Xiao, M Su and W Zhou. (2013). Clinical distribution and drug resistance of *Staphylococcus aureus* isolated from hospitalized children. Sichuan Da Xue Xue Bao Yi Xue Ban. 44:159-161.

Dormanesh B, F Safarpoor Dehkordi, S Hosseini, H Momtaz, R Mirnejad, MJ Hoseini, E Yahaghi, V Tarhriz and E Khodaverdi Darian. (2014). Virulence factors and o-serogroups profiles of uropathogenic *Escherichia coli* isolated from Iranian pediatric patients. Iran Red Crescent Med J. 16: e14627.

Efstratiou E, AI Hussain, PS Nigam, JE Moor , MA Ayub and JR Rao. (2012). Antimicrobial activity of *Calendula officinalis* petal extracts against fungi, as well as Gram-negative and Gram-positive clinical pathogens. Complement Ther Clin Pract. 18:173-6.

Faria RL, LM Cardoso, G Akisue, CA Pereira, JC Junqueira, AO Jorge and, PV Santos Júnior. (2011). Antimicrobial activity of *Calendula officinalis*, *Camellia sinensis* and chlorhexidine against the adherence of microorganisms to sutures after extraction of unerupted third molars. J Appl Oral Sci. 19:476-82.

Hartman D and JC Coetzee. (2002). Two US practitioners' experience of using essential oils for wound care. J Wound Care. 11:317-20.

Martins FS, EC da Conceição, ES Bandeira, JO Silva Junior and RM Costa. (2014). The effects of extraction method on recovery rutin from *Calendula officinalis* L. (Asteraceae). Pharmacogn Mag. 10:S569-73.

Momtaz H, A Karimian, M Madani, F Safarpoor Dehkordi, R Ranjbar, M Sarshar and N Souod. (2013). Uropathogenic *Escherichia coli* in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Ann Clin Microbiol Antimicrob. 29;12:8.

Muley BP, SS Khdabadi and NB Banarase. (2009). Phytochemical Constituents and Pharmacological Activities of *Calendula officinalis* Linn (Asteraceae): A Review. Trop J Pharm Res. 8:455-465.

Pan SY, SF Zhou, SH Gao, ZL Yu, SF Zhang, MK Tang, JN Sun, DL Ma, YF Han, WF Fong and KM Ko. (2013). New Perspectives on How to Discover Drugs from Herbal Medicines: CAM's Outstanding Contribution to Modern Therapeutics. Evid Based Complement Alternat Med, 2013: 627375.

Rigane G, S Ben Younes, H Ghazghazi and R Ben Salem. (2013). Investigation into the biological activities and chemical composition of *Calendula officinalis* L. growing in Tunisia. Int Food Res J. 20:3001-3007.

Rijal KR, N Pahari, BK Shrestha, AK Nepal, B Paudel, P Mahato and N Skalko-Basnet. (2008). Prevalence of methicillin resistant *Staphylococcus aureus* in school children of Pokhara. Nepal Med Coll J. 10:192-195.

Safarpoor Dehkordi F, S Barati, H Momtaz, SN Hosseini Ahari, S Nejat Dehkordi. (2013). Comparison of shedding, and antibiotic resistance properties of *Listeria monocytogenes* isolated from milk, feces, urine, and vaginal secretion of Bovine, Ovine, Caprine, Buffalo, and Camel species in Iran. Jundishapur J Microbiol. 6:284-94.

Tokajian S, D Haddad, R Andraos, F Hashwa and G Araj. (2011). Toxins and Antibiotic Resistance in *Staphylococcus aureus* isolated from a major Hospital in Lebanon. ISRN Microbiol. 2011: 812049.

Udo EE, N Al-Sweih, R Dha, TS Dimitrov, EM Mokaddas, M Johny, IA Al-Obaid, HH Gomaa, LA Mobasher, VO Rotimi and A Al-Asar. (2008). Surveillance of antibacterial resistance in *Staphylococcus aureus* isolated in Kuwaiti hospitals. Med Princ Pract. 17:71-75.

Virdis S, C Scarano, F Cossu, V Spanu, C Spanu and EP De Santis. (2010). Antibiotic Resistance in *Staphylococcus aureus* and coagulase negative *Staphylococci* isolated from goats with subclinical mastitis. Vet Med Int. 2010:517060.