International Journal of Biochemistry Research & Review

19(3): 1-7, 2017; Article no.IJBCRR. 37184
ISSN: 2231-086X, NLM ID: 101654445

Chemical Composition and Antibacterial Activity of *Cardamine uliginosa* Bieb. Growing Wild in Eastern Region of Turkey

Ömer Kılıç[1] and Fethi Ahmet Özdemir[2*]

[1]Department of Park and Garden Plants, Technical Science Vocational College, Bingol University, Bingol, 1200, Turkey.
[2]Department of Molecular Biology and Genetics, Faculty of Science and Art, Bingol University, Bingol, 1200, Turkey.

Authors' contributions

This work was carried out in collaboration between both authors. Author OK managed the analyses of the essential oil study, literature and plant sample searches. Author AO designed the study, performed the antibacterial analysis, wrote the protocol and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2017/37184
Editor(s):
(1) Shadaan Abid, Department of Internal Medicine, UT Southwestern Medical Center, Dallas, Texas, USA.
Reviewers:
(1) Hatice Taner Saraçoğlu, Selcuk University, Turkey.
(2) Miloslav Milichovsky, University of Pardubice, Czech Republic.
Complete Peer review History: http://www.sciencedomain.org/review-history/21565

Original Research Article

Received 3rd October 2017
Accepted 20th October 2017
Published 26th October 2017

ABSTRACT

Cardamine uliginosa Bieb. is a native plant belonging to Brassicaceae family which can used in ethnomedicine. This study was carried out to evaluate the essential oils composition of *Cardamine uliginosa* and its antibacterial activity. The plant sample was analyzed by GC/GC-MS system. Eventually twenty four components representing 91.2% of the total oil were identified. The obtained results proved the presence of twenty four components. The major component essential oil of this plant were identified as; limonene (32.6%), caryophyllene oxide (28.5%), β-caryophyllene (8.4%), *p*-cymene (3.4%), α-terpinolene (3.3%), β-pinene (2.4%), α-copaene (2.1%), α-longipinene (1.3%), δ-cadinene (1.2%) and β-ocimene (1.0%) was determined. The antibacterial activity of the essential oil was determined against sixteen bacterium isolates by measuring inhibition zones produced by the oil. The antibacterial activity of *C. uliginosa* essential oil was tested using the disc diffusion method wherein the essential oil has shown notably antibacterial effect with the inhibition zone in diameter from 2 mm (for *Pseudomonas aeruginosa* DSM 50070) to 11 mm (for *Bacillus subtilis* IM 622), with the exception of *Salmonella enterica* ATCC 1331 and *Salmonella typhimurium* NRRLE 4413 where

**Corresponding author: E-mail: ozdemirfethiahmet23@yahoo.com;*

the oils haven't shown antibacterial activity. Also weak inhibitory effect were observed against *Enterobacter aerogenes* CCM 2531, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSM 50070 and *Proteus vulgaris.* Chemical composition and antibacterial activity of the tested *Cardamine uliginosa* essential oil, obtained from plant material from the eastern region of Turkey, display a significant phytomedical potential.

Keywords: Cardamine uliginosa; essential oil; antibacterial activity.

1. INTRODUCTION

The Brassicaceae (Cruciferae), or mustard family, is a monophyletic group and includes more than 350 genera and 3500 species. Brassicaceae taxa generally cool season annuals, characterised by short cycle and wide adaptability; for this reason they are suited for cultivation in different seasons and in a variety of environments [1]. Also Cruciferae family includes many economically important ornamental and crop species (vegetables or sources of industrial and cooking oils, forage, and condiments). *Cardamine* L. is one of the largest genera of the Brassicaceae family, comprising at least 200 species distributed worldwide [1]. Both aerial parts and root provide the parts of some *Cardamine* species used in popular medicine to treat catarrhal ailments, respiratory tract, gastrointestinal tract and urogenital infections; and the infusion of this plant used as a mouthwash for mouth and larynx inflammation [2]. Some species of *Cardamine* have been consumed in the past as a salad and some as a remedy for scurvy [3]. The *Cardamine* species, commonly called toothworts have been used in folk medicine in different parts of the world as drugs for the treatment of toothache and as gastrointestinal aid [4]. Among them, *Cardamine diphylla* was very important for North American First Nations. The Algonquin gave an infusion of the plant to children with fevers, the cherokee gargled an infusion of the root to aid sore throats, the delaware used an infusion [3]. The root of *Cardamine* used as a stomach remedy and as a venereal aid and also as a remedy for the first stages of tuberculosis [3]. Moreover, the root of *Cardamine leucantha* has been included in a Chinese plant mixture for treating asthma [5]. Beside the medicinal uses, the decorative feature of *Cardamine* sp. makes them good candidates for landscape design [6]. *Cardamine flexuosa* has been shown to exhibit anticancer properties due to their high content of glucosinolates which hydrolyze to form bioactive products such as isothiocyanates [7].

The essential oil components depends on climatic, seasonal and geographic conditions, harvest period and distillation technique. In addition, their antimicrobial activity depends on the type, composition and concentration of the essential oils, the composition of the substrate and the processing and the storage conditions [8]. Usually, plant pathogenic fungi are checked up by synthetic fungicides but use of these is progressively limited because of the deleterious effects of pesticides on human health and the environment [9]. Plant metabolites, herbal-based medicines are think to be less harmful to human health as well as the environment compared to synthetic pesticides [10]. Excessive use of antibiotics has led to the ever more frequent occurence of resistant stems, especially in the case of first generation antibiotics [11]. For this reason, the last decades have seen massive efforts in the attempt to identify alternative treatments to the classical ways represented by antibiotic therapy. Plants have been used in traditional treatments to cure various diseases for thousands of years. Numerous studies have demonstrated that esssential oils obtained from plants represent antibacterial properties [12]. Particularly, the antimicrobial activities of plant essential oils and extracts have formed the basis of many applications including raw and processed food preservation, pharmaceuticals, alternative medicine and natural therapies [13].

There have been no previous reports on essential oil composition and antimicrobial activity studies on *Cardamine uliginosa*. The present study was aimed at identifying the essential oil composition of the *Cardamine uliginosa* and to determine antibacterial activities of this essential oil in an attempt to contribute to the use of these as alternative products for microbial control and food preservation.

2. MATERIALS AND METHODS

2.1 Plant Material

Plant sample was collected from their natural habitats in North of Floating Islands (Bingol-Solhan), *Quercus* forest openings, on 20.05.2016. Plant sample was identified by Kilic

with Flora of Turkey and East Aegean Islands [14]. The voucher specimens have been deposited at the Technical Vocational College, Department of Park and Garden Plants, Bingol University.

2.2 Isolation of the Essential Oils

The air-dried aerial parts of the *Cardamine uliginosa* was subjected to hydrodistillation using a Clevenger-type apparatus for 3-4 h, at the Technical Vocational College, Department of Park and Garden Plants, Bingol University (Turkey).

2.3 Gas Chromatography/ Mass Spectrometry (GC-MS) Analysis

A Varian 3800 gas chromatograph (GC), exactly interfaced with a Varian 2000 ion trap mass spectrometer (MS), was used with a splitless injection mode and an injector temperature of 260℃. The GC is equipped with 60 m long column packed with CP-Wax 52 CB 0.25 mm i.d. The oven temperature was 45℃ held for 5 min, then increased to 80℃ at a rate of 10℃ min^{-1}, and to 240℃ at a rate of 2℃ min^{-1}. Helium was the carrier gas which used at a stable pressure of 10 psi (Pounds per square inch); the transfer line temperature was 250℃; with an electron impact ionisation mode an acquisition range of 40 to 200 m z^{-1} and a scan rate of 1 us^{-1}. The compounds were identified using the NIST (National Institute of Standards and Technology) library, mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz [15]. The relative amounts were calculated on the basis of peak-area ratios. The identified constituents of the essential oils is listed in Table 1.

2.4 Evaluating Antibacterial Activity

2.4.1 Bacterial strains

In this study sixteen bacteria strains were used. The bacterial cells assayed included seven Gram-positive bacteria namely *Bacillus subtilis* ATCC 6337, *Brevibacillus brevis*, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IM 622, *Bacillus cereus* EMC 19, *Staphylococcus aureus* 6538 P, *Listeria monocytogenes* NCTC 5348 and the nine Gram negative bacteria *Salmonella typhimurium* NRRLE 4413, *Pseudomonas fluorescens*, *Enterobacter aerogenes* CCM 2531, *Klebsiella pneumoniae* EMCS, *Escherichia coli* ATCC 25922, *Proteus vulgaris* FMC II, *Pseudomonas aeruginosa* DSM 50070, *Proteus vulgaris*, *Salmonella enterica* ATCC 13311.

2.4.2 Antibacterial screening

The disc diffusion method was used for the determination antibacterial activity of essential oils. Suspension of the test bacteria strains in phase (100 µl) were spread on the solid Mueller Hinton Agar (Merck) media plates. Filter paper discs (6 mm in diameter, Bioanalyse) were individually impregnated with 10 µl of the essential oil and placed on the inoculated plates. Petri plates were placed at 4°C for 2 h. they were incubated at 37°C for 24 h. The diameter of the inhibition zones were measured in millimeters. Control disks with 20 µl DMSO showed no inhibition zone. All the tests were repeated triplicate.

3. RESULTS AND DISCUSSION

Essential oil composition of *Cardamine uliginosa* was analyzed by GC-MS revealed twenty four components representing 91.2% of the total oil were identified. All identified compounds percentages were given in Table 1. The major compounds were sesquiterpenes comprising up to 46.5%; and monoterpenes with 44.7% of the total oil composition (Table 1). Sesquiterpenes are the first main class and among these caryophyllene oxide (28.5%), β-caryophyllene (8.4%), followed by small percentages α-copaene (2.1%), α-longipinene (1.3%), δ-cadinene (1.2%) and others (Table 1). Monoterpenes are the second main class and among these limonene (32.6%), *p*-cymene (3.4%), α-terpinolene (3.3%), β-pinene (2.4%) followed by α-terpineol, α-pinene and others (Table 1). Among the monoterpenes, limonene was found to be the major constituent of studied sample. Among the detected compounds, caryophyllene oxide and β-caryophyllene (sesqiterpenes) were the main compounds of studied sample (Table 1). Based on presented results it can be concluded that limonene is the most abundant component (32.6%), followed by caryophyllene oxide (28.5%), β-caryophyllene (8.4%), *p*-cymene (3.4%), α-terpinolene (3.3%), β-pinene (2.4%), α-copaene (2.1%), α-longipinene (1.3%), δ-cadinene (1.2%), β-ocimene (1.0%) (Table 1). Essential oil of *Cardamine uliginosa* on growth of seven Gram-positive bacteria and nine Gram negative bacteria have been investigated. The results obtained in our study of antibacterial activity of the essential oils of *Cardamine uliginosa* are

shown on Table 2. To the best of our knowledge this is the first study reporting the antibacterial activity of *Cardamine uliginosa* essential oils. The essential oils have effect on fourteen bacterial strains, All of Gram-positive bacteria strains have affect of *Cardamine uliginosa* essential oils, while two Gram negative bacteria strains have not shown the response to the essential oil action (Table 2). *Cardamine uliginosa* essential oils have shown good inhibitory effect with the zone of inhibition in diameter of 2 mm (for *Pseudomonas aeruginosa* DSM 50070) up to 11 mm (for *Bacillus subtilis* IM 622), with the exception of *Salmonella enterica* ATCC 1331 and *Salmonella typhimurium* NRRLE 4413 where the oils haven't shown antibacterial activity. Also weak inhibitory effect were observed against *Enterobacter aerogenes* CCM 2531, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* DSM 50070 and *Proteus vulgaris* (Table 2).

In the literature, essential oil composition and antibacterial activity of essential oil *C. uliginosa* data is very lack and insufficient. With this study essential oil composition and antibacterial activity of *Cardamine uliginosa* essential oil will be enlarged. In this study limonene (32.6%) and caryophyllene oxide (28.5%) were found to be the main constituents of *C. uliginosa* (Table 1). According to studied results in the field of identifying the chemical composition in different species essential oil *Cardamine* genus, probably limonene, caryophyllene oxide and β-caryophyllene can introduced as the indicate components of this genus [16]. These findings supported the present study that limonene and caryophyllene are the most abundant compound in *C. uliginosa* essential oils.

The seeds of *Cardamine angulata* are rich sources of fatty acids [17]. The chemical consituents of *Cardamine* include alkaloids, flavonoids, essential oils, phenolic acids, terpenes, steroids, fatty acids, fatty acid methyl esters, triglycerides, amino acids, some other seconder metabolites, and elements [17]. *C. angulata* showed good antioxidant, antimicrobial and other biological activities [16]. The MeOH root extract of *Cardamine angulata* exhibited slight antibacterial activity (8.0-10.0 mm inhibition zone using a disc diffusion assay) against *Mycobacterium phlei* and methicillin-

Table 1. Essential oil composition of *Cardamine uliginosa*

	Compounds	*RRI	% Percentage
1	α-pinene	935	0.6
2	3-methyl-nonane	965	0.4
3	β-pinene	972	2.4
4	Myrcene	988	0.1
5	*p*-cymene	1020	3.4
6	Limonene	1030	32.6
7	β-ocimene	1042	1.0
8	α-terpinolene	1085	3.3
9	α-terpineol	1190	0.9
10	α-longipinene	1345	1.3
11	β-elemene	1385	0.6
12	α-copaene	1370	2.1
13	Dodecanal	1405	0.5
14	β-caryophyllene	1412	8.4
15	γ-elemene	1432	0.8
16	α-humulene	1448	0.2
17	β-farnesene	1453	0.3
18	α-selinene	1495	0.6
19	β-sesquiphellandrene	1520	0.9
20	δ-cadinene	1523	1.2
21	Caryophyllene oxide	1595	28.5
22	α-cadinol	1651	0.6
23	Bisabolol	1682	0.3
24	Ericosane	1698	0.2
		Total	**91.2**

**RRI: Relative Retention Index*

Table 2. Growth inhibition zones (mm) for *Cardamine uliginosa* essential oils

Bacteria	Gram	Inhibition zone diameter (mm)
Bacillus subtilis ATCC 6337	Positive	7
Brevibacillus brevis	Positive	9
Bacillus megaterium DSM 32	Positive	10
Bacillus subtilis IM 622	Positive	11
Bacillus cereus EMC 19	Positive	10
Staphylococcus aureus 6538 P	Positive	9
Listeria monocytogenes NCTC 5348	Positive	8
Salmonella typhimurium NRRLE 4413	Negative	-
Pseudomonas fluorescens	Negative	6
Enterobacter aerogenes CCM 2531	Negative	3
Klebsiella pneumoniae EMCS	Negative	4
Escherichia coli ATCC 25922	Negative	3
Proteus vulgaris FMC II	Negative	5
Pseudomonas aeruginosa DSM 50070	Negative	2
Proteus vulgaris	Negative	3
Salmonella enterica ATCC 13311	Negative	-
DMSO	Control	0

resistant *Staphylococcus aureus* [17]. The MeOH root extract of *C. angulata* displayed a slight inhibition zone 8.0–10.0 mm, using a disc diffusion assay) against the yeast *Saccharomyces cerevisiae* [18]. These results agreement with the present sutudy. The *Cardamine uliginosa* essential oils used in this study have affects all of tested bacterial strains. However, mantioned studies and the present study have got same differences related with inhibition zone diameter and chemical consituents. The reason for these differences are mostly used different species and different bacteria strains.

Various taxa of the *Cardamine* genus have antibacterial, antifungal, antimicrobial, antiviral, antioxidant, tyrosinase, nitrate reductase, phenylalanine ammonia lyase, peroxidase, and chitinase activities [16-18]. In addition, some *Cardamine* species have positive effects on diabetes and insects interact [19]. In the literature there are limited research about *Cardamine* species that have received little or no attention. Therefore, future chemical, antimicrobial, pharmacological, essential oil and toxicological studies on *Cardamine* taxa are of great importance; to huge potential of these species for medical applications could emerge. Essential oils are fairly complex mixtures. They generally show selective toxicity towards various pathogens and are relatively safe both to animals and humans. In complex mixtures, synergism of individual components is also expected so that microorganisms hardly can develop resistance towards essential oils. A large number of *in vitro* studies have shown a high antimicrobial power of essential oils [20-29]. Substantial number of previous studies has shown that resistance of gram negative bacteria is common and caused by a combination of factors, such as different cellular organization and poor permeability of the cell membrane, which acts as a barrier for antibacterial agents [30-31].

C. uliginosa essential oils evaluated in this study showed varying inhibitory activity on same tested microorganisms. It is worthwhile also to test other fractions for their antimicrobial activity potential. The *C. uliginosa* essential oils showed antibacterial activity, especially against Gram positive bacteria. Gram negative bacteria were found to be resistant to the *Cardamine uliginosa* essential oils. This study results being in line with specialty literature [32]. The antibacterial properties of the essential oils and their components have been studied in the past, the mechanism of their action has never been studied in detail [33]. Taking into account the large number of the different groups of chemical compounds present in the composition of the essential oils, it is very possible that their antibacterial activity can not be assigned to a sole mechanism, but to the existence of a large number of target locations in the cell. Not all these mechanisms represent separate targets; some are consequences of other target mechanisms [33,34]. The chemical structure of the individual compounds of essential oils affects in their specific ways the antibacterial action. It is therefore concluded that the studied plant species are the potential sources of essential nutrients along with significant phytomedicinal values. However, further researchs about

Cardamine species are required to isolate the individual constituents responsible for the antioxidant, antimicrobial and biological activities and find their applications in food and pharmaceutical industries [35].

4. CONCLUSIONS

In this study qualitative and quantitative differences were found studied *Cardamine* species in view of compounds depending on genetic, environmental factors, ontogeny, season, plant part analyzed and analytical methods. The findings showed that the genus *Cardamine* had a considerable variation in essential oil composition and this study demonstrates the occurrence of the limonene/caryophyllene oxide and β-caryophyllene chemotype in the eastern Anatolian region of Turkey. Moreover significant results were obtained about essential oil and antibacterial evaluation of the *Cardamine* taxa.

ACKNOWLEDGEMENTS

The authors acknowledge the Scientific and Research Council of Bingol University (BAP - TBMYO.2016.00.001) for support this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Shehbaz IA, Beilstein MA. Systematics and phylogeny of the brassicaceae (Cruciferae): An overview. Plant Syst. and Evol. 2006;259:89-120.
2. Guarino C, Simone L, Simona S. Ethnobotanical study of the sannio area, campania, Southern Italy. Ethnobot. Res. & App. 2008;6:255-317.
3. Moerman DE. Native American ethnobotany, Timber Press, Portland. 1998;137.
4. Sturtevant LE. Sturtevants edible plants of the world, Ed. U. P. Hedrick, Dover Publications, New York. 1972;140.
5. Gao PR. China Pat; 2011 (in Chinese).
6. Saribas M, Kaya Z, Baran S, Yaman B, Sabaz M. The use of some natural plant species from the Western Black Sea Region of Turkey for landscape design. Fresenius Environ. Bull. 2007;16:193-200.
7. O'Hare TJ, Wong LS, Force LE, Williams DJ, Gurung CB, Irving DE. Glucosinolate composition and anti-cancer potential of daikon and radish sprouts. Acta Hort. 2008;765:237-244.
8. Marino M, Bersani C, Comi G. Impedance measurements to study the antimicrobial activity of essential oils from lamiaceae and compositae. Int. J. Food Microbiol. 2001;67:187–195.
9. Harris CA, Renfrew MJ, Woolridge MW. Assessing the risk of pesticide residues to consumers: Recent and future developments. Food Add. and Cont. 2001; 18:1124-1129.
10. Kordali S, Çakır A, Akcin TA, Mete E, Akcin A, Aydın T, Kılıç H. Antifungal and herbicidal properties of essential oils and n-hexane extracts of *Achillea gypsicola* and *Achillea biebersteinii*. Indust. Crops and Prod. 2009;29:562-570.
11. Vancanneyt M, Zamfir L, Devriese A, Lefebvre K, Engelbeen Vandemeulebroecke K, Amar M, De Vuyst L, Haesebrouck F, Swings, J. *Enterococcus saccharominimus* sp. nov., from dairy products. Int. J. Syst. Evol. Microbiol. 2004;54:2175-2179.
12. Yoneyama H, Katsumata R. Antibiotic resistance in bacteria and its future for novel antibiotic development. Biosci. Biotechnol. Biochem. 2006;70:1060-1075.
13. Lis-Balchin M, Deans SG. Bioactivity of selected plant essential oils against *Listeria monocytogenes*. J. Appl. Bacteriol. 1997;82:759–762.
14. Davis PH. Flora of Turkey and East Aegean Islands. University Press, Edinburgh. 1982;7.
15. Van den Dool H, Kratz PD. A generalization of the retention index including linear temperature programmed gasliquid chromatography. J. of Chromatog. 1963;11:463-471.
16. Drozdova IV, Alexeeva-Popova N. V. Bot. Zh. (Sankt-Petersburg). Types of mineral metabolism of the plants of Southern Chukotka. Russ. J. of Ecol. 1998;7:53-58.
17. Sabine M, Rene S. Bleeker. *Cardamine* sp. - A review on ıts chemical and biological profiles. Chem. & Biodiversity. 2011;8:955-975.
18. McCutcheon AR, Ellis SM, Hancock REW, Towers GHN. Antifungal screening of medicinal plants of British Columbian native peoples. J. Ethnopharm. 1994;44: 157-169.

19. Kvetensky J, CasLek Ces. Preliminary report on the treatment of diabetes using the plant Cardamine opizii presl. Cas Lek Cesk. 1967;106:163-165.
20. Miletic P, Marjanovic-Balaban Z, Kalaba V. Antimicrobial activity of essential oil from Norway spruce (*Picea abies*). VIII Symposium "Advanced Technology and Economic Development". Proceedings, Leskovac. 2009;8:1-9.
21. Kalaba V, Marjanovic-Balaban Z, Stijepic M, Glušac J, Kalaba D. Antimicrobial activity of selected essential oils against of *Staphylococcus aureus* compared with antimicrobial drugs. II International Congress Food Technology Quality and Safety, Novi Sad, Proceedings. 2014;434-439.
22. Nikolic M, Glamodzlija J, Ciric A, Markovic T, Markovic D, Peric T, Sokovic M. Chemical composition and antimicrobial activity of essential oil from mint (*Mentha piperita* L.). Lek. Sirov. 2013;33:63-72.
23. Mahmmod ZA. The effect of chamomile plant (*Matricaria chamomile* L.) as feed additives on productive performance, carcass characteristics and immunity response of broiler. Int. J. Poult. Sci. 2013; 12:111-116.
24. Jakubcova Z, Zeman L, Horky P, Mrkvicova E, Mares P, Mrazkova E, Stastnik O. The influence of the addition of chamomile extract to the diet of chickens. Proceedings of the Conference Mendel Net, Brno, Czech Republic. 2014;147-150.
25. Jakubcova Z, Zeman L, Mares P, Mlcek J, Jurikova T, Dostalova L, Mrazkova E, Mrkvicova E, Balla S, Sochor J. Effect of chamomile supplements to feeding doses on antimicrobial parameters in poultry. Potravinarstvo. 2014;8:228-232.
26. Al-Mashhadani EH, Al-Mashhadani H, Al-Shamire JS. Effect of supplementing different levels of chamomile oil on broiler performance and some physiological traits. Int. J. Poult. Sci. 2013;12:426-429.
27. Beatovic DV, Jelaèic SC, Oparnica CD, Krstic-Miloševic DB, Glamodzlija JM, Ristic MS, Šiljegovic JD. Chemical composition, antioxidative and antimicrobial activity of essential oil of *Ocimum sanctum* L. Hem. Ind. 2013;67: 427-435.
28. Mitrovic SV, Randelovic NV, Ristic MS, Dimic M. Chemical composition and antimicrobial activity of essential oils from *Mentha longifolia* and *M. aquatic* L. 7th symposium on flora of Southeastern Serbia and Neighbouring Regions. 2002; 99-102.
29. Stanojevic LP, Marjanovic-Balaban ZR, Kalaba VD, Stanojevic JS, Cvetkovic DJ. Chemical composition, antioxidant and antimicrobial activity of chamomile flowers essential oil (*Matricaria chamomilla* L.). Teop. 2016;19:2017-2028
30. McDonnell G, Russell AD. Antiseptics and disinfectans: Activity, action and resistance. Clin. Microbiol. Rev. 1999;12: 147-179.
31. Lambert PA. Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. J. R. Soc. Med. 2002;95:22-26.
32. Directive. European Parliament and of the council of 18 September 2000. On the protection of workers from risks related to exposure to biological agents at work. Official Journal. 2000;262:21–45.
33. Arai R, Sugita T, Nishikawa A. Reassessment of the *in vitro* synergistic effect of fluconazole with the non–steroidal antiinflammatory agent ibuprofen against *Candida albicans.* Mycoses. 2005; 48:38-41.
34. Chatterjee A, D'Souza D, Vira T. Strains of *Mycobacterium tuberculosis* from Western Maharashtra, India exhibit a high degree of diversity and strain–specific associations with drug resistance, cavitary disease and treatment failure. J. Clin. Microbiol. 2010;48:3593-3599.
35. Khan H, Jan SA, Javed M, Shaheen R, Khan Z, Ahmad A, Safi SZ, Imran M. Nutritional composition, antioxidant and antimicrobial activities of selected wild edible plants. J. of Food Biochem. 2015; 4:61-70.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/21565