



Evaluation of Antimicrobial Activity of the Aquatic Extract against Bacterial isolates from URTI in Babylon Province, Iraq

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Abstract

Background, The other infections of patient in the hospitals may be occur resulting from high drug resistance bacteria and causes mortality. The study have been aimed to investigate the antibacterial activity of aqueous extract *Borago officinalis* L., *Cydonia oblonga*., *Matricaria chamomilla* against most prevalence pathogenic bacteria. Methods; the aqueous extract *Borago officinalis* L., *Cydonia oblonga*., *Matricaria chamomilla* were used to investigate the antibacterial activity pathogenic bacteria by Agar well diffusion method. Result; the Result showed that the crude of all plants extract have potential antibacterial activity. Conclusion; Based on the results it can be concluded that plants extract can inhibit bacterial colonization and adherence to upper respiratory tract infection, and provide production against different human pathogens and this may have clinical relevance.

Keyword: *Borago officinalis* L, *Cydonia oblonga*, *Matricaria chamomilla*, Pathogenic bacteria.

Introduction

The Resistant to the drug by microorganisms has increased in the last three decades, contributed to A new bacterial strain which is multi-resistance. The other infections of patient in the hospitals may be occur resulting from high drug resistance bacteria and causes mortality (1). The plants Medicinal are finding use as and pharmaceuticals, nutraceuticals. In the centuries; the product of Plant derived has been used for the medicinal purposes. Due to the side effect of antibiotics.

The new resources for microbial agents that could act as alternatives to drug (antibiotics) in the treatment of diseases. The medical plant is considered one of the important reasons for the success treatment antibiotic-resistant bacterial infection (2).

The Borage (*Borago officinalis* L.) plant belong to *Boraginaceae* family is spreads in many Mediterranean countries. Borage used for many folk medicinal purposes (3), and as well as for preparing of salads and beverages (4). Plants and seeds of borage provide bio-

active compounds (5), contributed to the active component of this plant to acids of gamma linolenic (GLA) (6). the borage are gaining increasing agricultural interest (7).

Another an important herbal that is produce secondary products or metabolites that are biosynthetically derived from first or primary products or metabolites and components the important of source of several antibiotics are *Cydonia oblonga* (8). *Cydonia oblonga* called Quince are rich in useful secondary product like flavonoids, steroids, terpenoids, tannins, organic acids, phenolic, glycosides and sugars (9).

Quince are used to treat or prevent many ailments like hepatitis, diabetes, cancer, ulcer, urinary, infection of respiratory (10) and the plant of medical is safe. furthermore, the toxic affectivity of the Quince produced by its seeds of plants when the man are ingested the increase amounts of Quince contributed to presence of components of nitrile (11, 12).

The flower-head of *Matricaria chamomilla* (Chamomile) are contained many active compounds of groups having important therapeutic (active compounds) values essential oil and sesqui-terpene (13). *Matricaria chamomilla* secreted the Essential oils could be employed as antibacterial agents (14). Moreover, potential sources of novel antimicrobial active compounds especially against bacterial organism and the plants have highest anti-parasitic, antifungal, antibacterial, antiviral, spasmolytic and antioxidant activity (15).

Matricaria chamomilla are called medicinal plants belong family to the Asteraceae often referred to the "star among medicinal plant (16). The study have been aimed to investigate the antibacterial activity of aqueous extract *Borago officinalis* L., *Cydonia oblonga.*, *Matricaria chamomilla* against most prevalence pathogenic bacteria.

Material and Method

Preparation of Plants Extract

The Aqueous preparation were collected from Al- Hilla in 2017 from the retail food store . Then 30 gram of powder of extract was soaked by 100 ml of water (distillated water), left to stand (3days) and filtration to sterilization by (diameter 0.45 paper of Millipore filter). This solution of extract were 30% of concentration of this plant extract (17).

The Isolates of Microorganism

Eight and three of Gram-negative, Gram-positive isolates respectively (table 1) were isolated from the patients were suffering from respiratory infections in the AL-Hashmyia teaching hospital (department of infectious diseases). The microorganisms were identify by the conventional biochemical tests, the identification was confirmed by using Api 20 E, Api 20 NE of biochemical galleries, and minivans apparatus. These organisms was confirmed by using conventional biochemical tests. The microorganisms were activated, culturing and cloned on the nutrient agar (three times) (18).

Table 1 Bacterial Isolates	
Gram positive isolates	Gram negative isolates
<i>S. aureus</i>	<i>P. aeruginosa</i>
<i>S. epidermidis</i>	<i>P. fluresence</i>
<i>S. saprophyticus</i>	<i>P. vulgaris</i>
	<i>K. pneumoniae</i>
	<i>E. aerugenes</i>
	<i>E. coli</i>
	<i>Proteus mirabilis</i>
	<i>Acinetobacter</i>

Antimicrobial Effect by Agar well Diffusion Methods; An *In vitro* (19)

The methods of agar well diffusion were used for determined the antibacterial activity. The growth of Loopfull from organism was inoculated and incubated into broth media (nutrient), incubated at 37 °C for 1 day respectively.

The bacterial suspensions were diluted with (normal saline). then the turbidity was compare with (number 0.5) of McFarland standard-tube to yield the suspension of uniform which containing (1.5×10^8 CFU / ml). A Dip swab cotton into adjustment microbes suspension and streak the entire the surface of plates media (Mueller-Hinton agar and sabouraud dextrose agar) and the cultured plates were left for 10 minutes at 25

C° to dry. the cultured Media were cut by the cork borer, the diameter of wells were 6mm (five wells in petri dish) and add 0.6 ml of the aquatic extracts. The cultured plates were putted overnight in the incubator at 37 C°. A size of inhibition zone of was measured from edge of well to the edge of growth of inhibition.

Antibacterial Activity Assay

The agar of ciprofloxacin disks diffusion were used for determined the antibacterial activity according to [20].

Result and Discussion

The results of current study clarified that the extract of *B. officinalis* could be used successfully in the damage of bacterial infections like urinary tract, respiratory system, skin diseases (Figure 1)

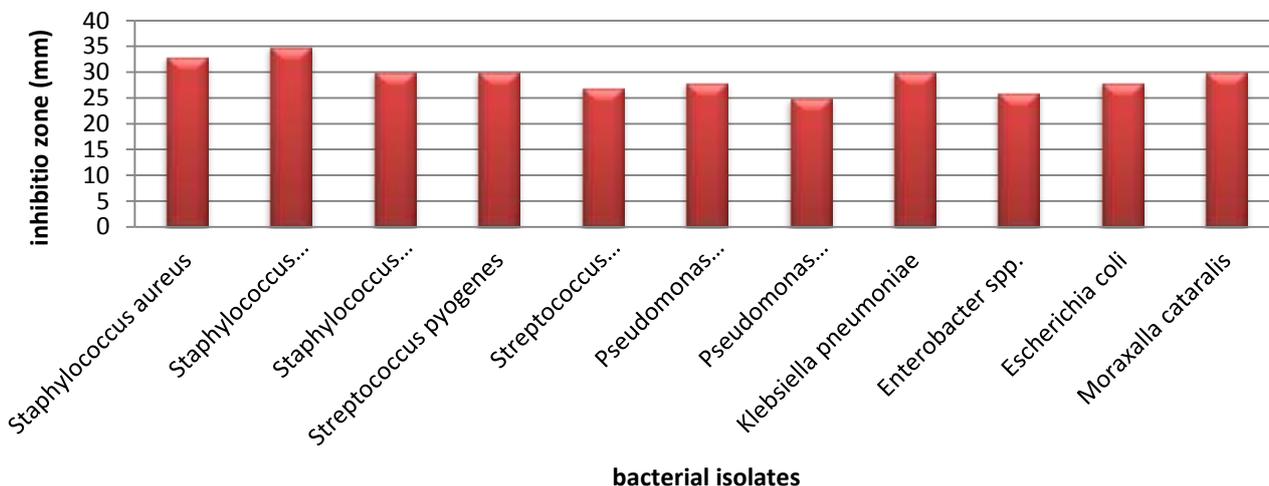


Figure 1: Antibacterial activity of *Borago officinalis* against bacterial isolates

Some studies indicated that analysis with useful the gas chromatography-mass-spectrometry (GC-MS) in the oil seed extracts of *B. officinalis* appears sixteen compounds of volatile include; small amounts of 0.7% nonadecane, 19.7% of p-cymene-8-oil, 0.7% hexanol, and 26% of β -caryophyllene, whereas large amounts of oil monoterpenes were 17.2% and 26% of sesquiterpenes. On the other hand, the fatty acids have been isolated like 35-40% linoleic acid, 10–28% of γ -linolenic acid, and 4–5% α -linolenic acid. In the seeds extract of the presence of the acids of rosemary in the amount of 1.65 mg/g from dry weight have been measured (21). Moreover, the gamma-linolenic acid (GLA), that are responsible for many of the pharmacological effects (22).

In the other hand, the antibacterial activity of aqueous extract of *Borago officinalis* was high against bacteria due to the presence of tannins and flavonoids (23). In vitro methods clarified of the aqueous extract were inhibited the growth of species of *Staphylococcus* and this is also documented by (24). Flavonoid of extracts have good antimicrobial effect against microorganism, while the different phenolic explored in seeds of Quince include; 3,5-

dicafeoyl quinic acid and the acids of 5-O-cafeoyl quinic, apigenin derivatives (vicenin-2, isoschaftoside, and schaftoside), 6-C-glucosyl-8-C-pentosyl chrysoeriol, and stellarin-2 leucenin-2, 6-C-pentosyl-8-C-glucosyl chrysoeriol (25). A flavones is the major part of constituents of active components of phytochemical caffeoylquinic acids (35–37%), (63–66%) with isoschaftoside, and (19–24%) of 5-O-cafeoylquinic acid as prominent flavones compounds.

The acids of the Organic of extracts seeds in the Quince are identified as L(shikimic, fumaric, D-quinic, ascorbic, citric, and malic acids). Moreover, The acids of amino identified in the seeds (freeze dried) were L of (proline, valine, glycine, alanine, glutamic acid, isoleucine, cysteine, leucine, hydroxyproline, serine, asparagine, phenyl alanine, threonine, tryptophan, methionine, aspartic acid, ornithine, histidine, glutamine, and tyrosine), which constitute sample about 1.3–1.7 mg/kg of sample. Furthermore, L of (glutamic acid, aspartic acid, and asparagine) were 60–75% from the total amino acids (26). (Figure 2) reveals the antibacterial action of seed of *Cydonia oblonga* against tested bacterial isolates

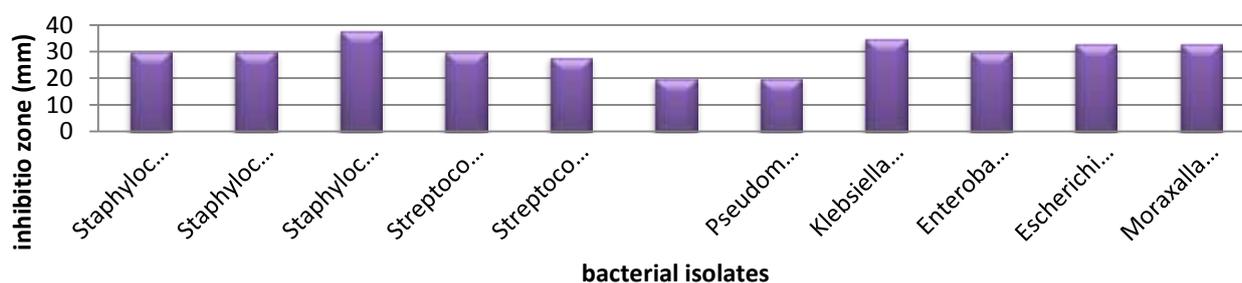


Figure 2: Antibacterial activity of seed of *Cydonia oblonga* against bacterial isolates

In this figure higher antibacterial action are obtained in *Staphylococcus saprophyticus* 38mm followed by *Klebsiella pneumonia* 35mm then 33mm against *Escherichia coli*, *Moraxalla cataralis* and 30mm in *Streptococcus pyogenes*, *Enterobacter spp* while 20 mm in *Pseudomonas aeroginosa* and *Pseudomonas fluroscences*. Finally 28 mm are obtained against *Streptococcus pneumonia*.

The results reveals that the bacteria of gram-negative were very resistant and more than gram positive bacteria. Due to the resistance of bacteria (gram negative) may be related to the cell wall like lipopolysaccharides in their outer membrane of organism (27).

The seed plant extract of *Cydonia oblonga* have been reported to have sterols, triterpenoic acids phytofluene, and carotenoids phytoene (28), phenolic compounds (29), organic acids such as shikimic, ascorbic, citric, fumaric, malic and quinic acids, whereas the free amino acids

were; glutamic, the aspartic, and asparagines, are the most abundant (30). Similarly, from the fatty acids, linoleic, the oleic, palmitic and capric acids are the major constituents comprising around 85.06 % of its oil (31).

Tannins has been showed to prevent the development of organisms by the protein precipitating microbial and making protein nutritiona unavailable for them (32). It therefore suggests that the seeds of extract used in the present study may has a selected antibacterial effects. The largest size of the inhibition zones indicated the potency of the active component principles of the seeds extract.

The results of current study show that *Matricaria chmomilla* may be used successfully in the adjunctively in disorders or damage of the bacterial infection of skin diseases, urinary tract and respiratory system (Figure3).

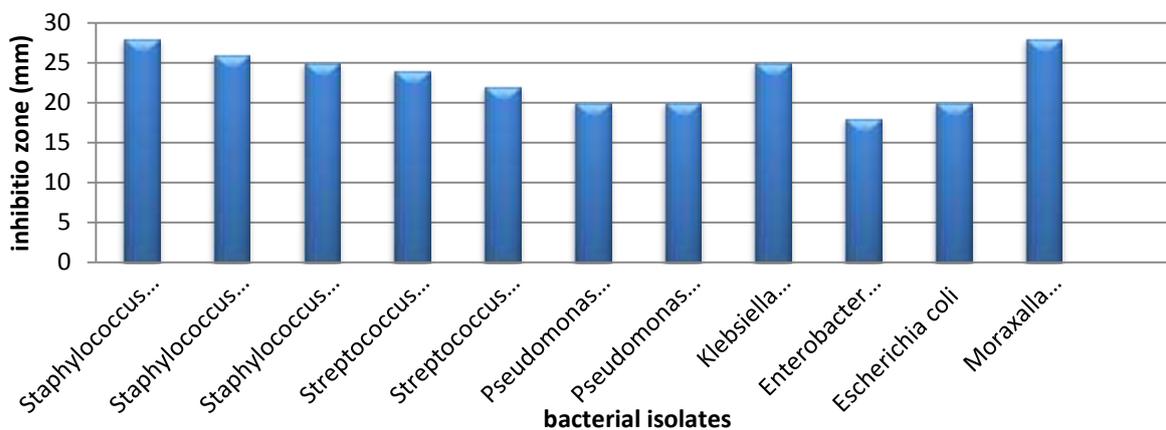


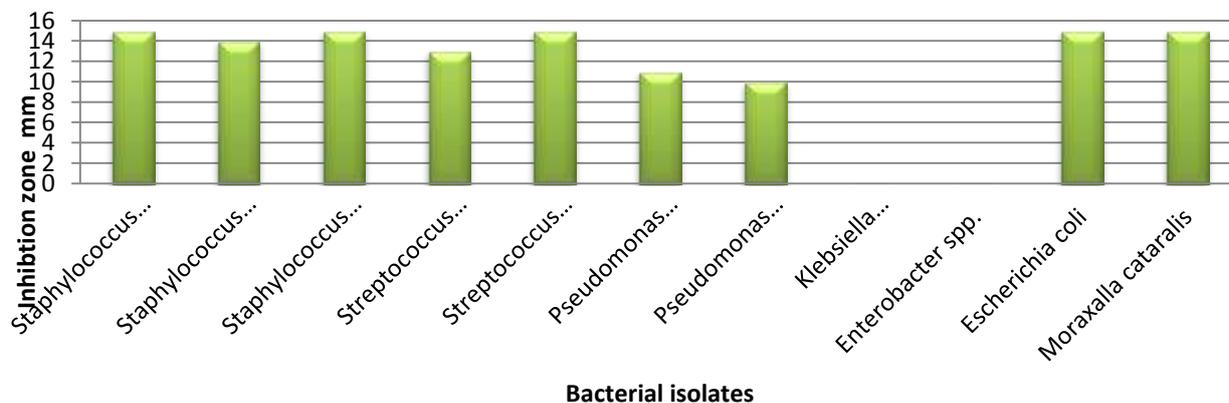
Figure 3: Antibacterial activity of *Matricaria chmomilla* against bacterial isolates

Some studies ensured that phenolic compounds (flavonoids and tannins) have high antibacterial activity because they contain hydroxyl group (-OH) in their chemical structure which it has ability to bonding with proteins hydrogen and this leads to break of sulphuric and hydrogen bonds abundant in the tertiary structure of proteins existing in bacterial cell (33).

Also the phenols are capable of destruction of cell wall then increase of its permeability for these compounds leading to denaturation of cell proteins (34).Some studies indicated that the phenols have ability to bind with cell enzymes leading to inhibition of its biochemical activity (35).

The inhibition diameters values were greater towards positive bacteria than negative bacteria because *Escherichia coli* contains dense lipid layers in its cellular wall leading to resistance the entrance of the phenolic compounds into the bacteria cell . This biochemical case is opposite to *Staphylococcus aureus* which contains less lipid layers.

In this search we also compare between the result obtained by tested extract and results obtained by ciprofloxacin antibiotic(as most traditional treatment for infection caused by tested bacteria) concerning their antimicrobial activity against tested bacteria; as reveals in Figure (4):



aqueous extracts showed more activity as antimicrobial agent against tested bacterial isolates compare to ciprofloxacin according to inhibition zone diameter and even antibiotic resistant strains (*Pseudomonas aeruginosa*, *Pseudomonas fluorescences*, *Klebsiella pneumoniae*) and less sensitivity bacteria to ciprofloxacin (*Staphylococcus aureus*, *Staphylococcus epidermidis*). These bacterial strain is a mainly multi-drug resistant

bacteria that are most commonly found, especially through nosocomial infections (36).

Considering the findings of this study and comparison with other studies in this field tea extract can be controlled growth of ciprofloxacin resistance bacterial strains which involved in this study within vitro condition.

References

- Cohen, M.L. Epidemiology of drug resistance: implications for a post-antimicrobial era. *Science* 257, 1050-1055, 1992
- Tepe B, Daferera D, Sökmen M, Polissiou M, Sökmen A. In-vitro antimicrobial and antioxidant activities of the essential oils and various extracts of *Thymus eigi* M. Zohary et P.H. Davis. *J Agric Food Chem.* 2004; 52:1132–7.
- Hassan Gilani, A., Bashir, S., and Khan, A. (2007). Pharmacological basis for the use of *Borago officinalis* in gastrointestinal, respiratory and cardiovascular disorders. *Journal of Ethnopharmacology*, 114, 393e399.
- Branca, F. (2001). Trials related to the cultivation of wild species utilized in Sicily as vegetables. *Italus Hortus*, 8, 22.
- Bandoniene, D., & Murkovic, M. (2002). The detection of radical scavenging compounds in crude extract of borage (*Borago officinalis* L.) by using an on-line HPLC-DPPH method. *Journal of Biochemical and Biophysical Methods*, 53, 45e49.
- Mhamdi, B., Wannes, W. H., Bourgou, S., & Marzouk, B. (2009). Biochemical characterization of borage (*Borago officinalis* L.) seeds. *Journal of Food Biochemistry*, 33, 331-341.
- El Hafid, R., Blade, S. F., & Hoyano, Y. (2002). Seeding date and nitrogen fertilization effects on the performance of borage (*Borago officinalis* L.). *Industrial Crops and Products*, 16, 193e199.
- Al-Snafi AE. Therapeutic properties of medicinal plants: a review of their effect on reproductive systems (part 1). *Ind J of Pharm Sci & Res* 2015; 5(4): 240-248.
- Aksic, M. F., Tosti, T., Nedic, N., Markovic, M., Licina, V., Milojkovic-Opsenica, D., et al. (2015). Influence of frost damage on the sugars and sugar alcohol composition in quince (*Cydonia oblonga* Mill) floral nectar. *Acta Physiol. Plant.* 37, 1–11.
- Ahmed, M. M., and Bastawy, S. (2014). Evaluation of anti-inflammatory properties and possible mechanism of action of Egyptian quince (*Cydonia oblonga*) leaf. *Egypt. J. Biochem. Mol. Biol.* 32, 190–205.
- Al-Snafi AE. Therapeutic properties of medicinal plants: a review of plants with anticancer activity (part 1). *Int J of Pharmacy* 2015; 5(3): 104-124.
- Hamauzu, Y., Irie, M., Kondo, M., and Fujita, T. (2008). Anti-ulcerative properties of crude polyphenols and juice of apple and Chinese quince extracts. *Food Chem.* 108, 488–495.
- Gosztola, B., Sarosi, S., and Nemeth, E., (2010). Variability of the Essential Oil Content and Composition of Chamomile (*Matricaria recutita* L.) affected by Weather Conditions. *Nat. Prod. Comm.*, 5: 465–470.

- 14 Sefidkon F and Ahmadi, S.H. (2000). Essential oil of *Tanacetum pathenium* L. *J. Essent. Oil Res.*, 12: 427–428
- 15 Prabuseenivasan, S., Jayakumar, M., and Ignacimuthu, S., (2006). In vitro antibacterial activity of some plant essential oils. *BMC Complementary Altern. Med.*, 6: 39
- 16 Franz Ch, Bauer R, Carle R, Tedesco D, Tubaro A, Zitterl-Eglseer K. Study on the assessments of plants/herbs, plant/herb extracts and their naturally or synthetically produced components as additives for use in animal production. CFT/EFSA/FEEDAP/2005/01. 2005: 155–69.
- 17 Hindi NKK, Al-Mahdi Z K A, Chabuck ZAG. Antibacterial activity of the aquatic extract of fresh, Dry powder ginger and crud oil of ginger (*Zingiber officinale*) against different types of bacteria in Hilla city, Iraq. *Int J Pharm Pharm Sci*, 2014 6(5) 414-7.
- 18 Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scotts' Diagnostic microbiology. 12th ed. Elsevier. China.2007.
- 19 NCCLS (National Committee for Clinical Laboratory Standards). Methods for dilution antimicrobial susceptibility tests of bacteria that grow aerobically. Approved Standard M100-S12. Wayne, PA, NCCLS.2000.
- 20 Hindi NKK, Al-Mahdi Z K A, Chabuck ZAG. Antibacterial activity of the aquatic extract of fresh, Dry powder ginger and crud oil of ginger (*Zingiber officinale*) against different types of bacteria in Hilla city, Iraq. *Int J Pharm Pharm Sci*, 2014 6(5) 414-7.
- 21 Mhamdi B, Wannas WA, Bourgou S, Marzouk B. Biochemical characterization of borage (*Borago officinalis* L.) seeds. *J Food Biochem* 2009; 33:331-41.
- 22 Ziboh, V. A., Naguwa, S., Vang, K., Wineinger, J., Morrissey, B. M., Watnik, M., and Gershwin, M. E. Suppression of leukotriene B4 generation by ex-vivo neutrophils isolated from asthma patients on dietary supplementation with gammalinolenic acid-containing borage oil: possible implication in asthma. *Clin Dev Immunol* 2004;11(1):13-21. E. Van der Watt and J. C. Pretorius, "Purification and Identification of Active Antibacterial Components in *Carpobrotus edulis* L.," *Journal of Ethnopharmacology*, Vol. 76, No. 1, 2001, pp. 87-91.
- 23 Miceli A., Aleo A., Corona O., Sardina M.,T., Mammia C., Settanni L. (2014). Antibacterial activity of *Borago officinalis* and *Brassica juncea* aqueous extracts evaluated in vitro and in situ using different food model systems. *Food Control*.(40),157-164
- 24 Anwar F., Muhammad G., Hussain M. A., Zengin G., Alkharfy K. M., Ashraf M., et al. (2016). *Capparis spinosa* L.: a plant with high potential for development of functional foods and nutraceuticals/pharmaceuticals. *Int. J. Pharmacol.* 12, 201–219. 10.3923/ijp.2016.201.219
- 25 Silva B. M., Andrade P. B., Ferreres F., Seabra M. R., Oliveira M. B. P. P., Margarida A. F. (2005). Composition of Quince (*Cydonia oblonga* Miller) seeds: phenolics, organic acids and free amino acids. *Nat. Prod. Res.* 19, 275–281.
- 26 Gao Y, Belkum MJ, Stiles M (1999). The outer membrane of Gramnegative bacteria inhibits antibacterial activity of Brochacin C. *Applied Environ. Microbial.* 65(4329-4333).
- 27 Lorenz, P., M. Berger, J. Bertrams, K. Wende, K. Wenzel, U. Lindequist, U. Meyer, and F.C. Stintzing (2008). Natural wax constituents of a supercritical fluid CO2 extract from quince (*Cydonia oblonga* Mill.). *Anal. Bioanal. Chem.* 391: 633-646.
- 28 Oliveira, A. P., J. A. Pereira, P. B. Andrade, P. Valentão, R. M. Seabra and B. M. Silva (2007). Phenolic smooth muscle. *Rev. Pharmacol. Toxicol.* 17: 149-166.
- 29 Silva, B.M., P.B. Andrade, F. Ferreres, R.M. Seabra, M.B.P.P. Oliveira, and M.A. Ferreira (2005). Composition of quince (*Cydonia oblonga* Miller) seeds: Phenolics, organic acids and free amino acids. *Nat. Prod. Res.* 19: 275-281
- 30 Turkoz, S., S. Kusmenoglu , and U. Koca (1998). Studies on the seeds of *Cydonia oblonga* Miller. *Acta Pharm. Suec.* 40: 39-42.
- 31 Fluck H (1973). Medicinal Plants and their uses. W. Feulsham and Co.Ltd, New York. pp. 7-15.
- 32 J . Collee , A . Fraser , B . Marimion and A . Bimon , Practical medical microbiology , Makie and Mc. Carteney , 4th ed. , Churchill Livingston , New York , 978 , (1996) .
- 33 P . Feeny , Inhibitory effect of Oak Leaf Tannins on the Hydrolysis of proteins by Trypsine , J . Phytochemistry , 8 , 2116 , (1998) .
- 34 R. L. Lindroth and M. S. Bloomer, Biochemical ecology of foresttent caterpillar response to dietary protein and phenolic glycosides , J . Env. Emtocol. , 86, 408-413 , (1991) .
- 35 Adcocks C., Collin P., Buttle D. J. (2002). Catechins from green tea (*Camellia sinensis*) inhibitbovine and human cartilage proteoglycan and type II collagen degradation in vitro. *J. Nutr.* 132, 341–346