Original Article

Comparison of Rosa Nutkana Sepal Extract with Synthetic Antibiotics for Treatment of Methicillin Resistant *Staphylococcus Aureus* Isolated from Patients with Sty

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Abstract

Objective: This research was carried out to compare antibacterial effect of Rosa nutkana sepal extract with synthetic antibiotics on methicillin resistant staphylococcus aureus (MRSA) bacteria isolated from patients with sty.

Patients and Methods: This descriptive study was done in 2009 at remedial center of Shahid Beheshti University and sampling was performed from patients suffering from sty using sterile soap. After preparation of Rosa nutkana sepal extract and after determining bacteria type, antibiogram test was used to determine bacterial resistance and sensitivity to cephalothin, co-amoxiclav, amoxicillin and tetracycline, as well as to the extract. Disc diffusion and agar dilution were used for this purpose. The area of non-growth resulting from antibiotic discs was compared with of the extract.

Results: In this study, 31% of samples were reported to be MRSA. MRSA in 1/128 concentration of extract was sensitive in minimum inhibitory concentration (MIC) test.

In disc diffusion test, Staphylococcus aureus was 100% sensitive to the extract, 15.8% to cephalothin, 26.3% to tetracycline, 31.6% to co-amoxiclav and 100% resistant to amoxicillin.

Furthermore, there was not any microbial growth in agar dilution method (nutrient agar environment and 0.01% extract of plant sample). Moreover, there was not any microbial growth in plates containing *Pseudomonas aeruginosa* and *Candida albicans*.

Conclusion: Rosa nutkana sepal extract with antibiotics can be an important combination for treatment of sty infection.

Key words: Extract, Sepal, Rosa nutkana, Resistance, Sensitivity.

Introduction

A look over medical products market in recent decades shows the popularity of some products with herbal source, so producers tend to put herbal label on some parts of their products. New findings in this science have provided a lot of services in medical industry some of which is presented here: herbal derivations have various remedial effects and sometimes a plant can have some simultaneous effects and substitute several chemical substances (1).

Consumers have a favorable view regarding herbal products, because they know it as a natural product which certainly has fewer side effects. The only disadvantage of these products is that formulization of herbal products is difficult and maybe the biggest problem in this industry. (2, 3)

Therefore in a descriptive study in 2009 on methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from patients suffering from sty, at the remedial center of Shahid Beheshti University, the antibacterial power of Rosa nutkana sepal extract was evaluated comparing with synthetic antibiotics.

Staphylococcus is a genus of spherical gram-positive bacteria which forms in grape-like clusters. They become resistant to anti-bacterial agents rapidly and cause lots of remedial problems.

Staphylococcus includes at least 30 species. From clinical point of view, however, there are three important species: *staphylococcus aureus, staphylococcus epidermidis,* and *staphylococcus saprophyticus.*

staphylococcus aureus is coagulase positive which distinguishes it from other species. It is an important pathogen for human. Almost every human being experiences some types of staphylococcus infection such as food poisoning, slight skin infections to life threatening widespread infections. (4, 5, 6)

Sty is a common disease affecting patients in different ages. It appears like an inflated, sensitive, painful and red lump near the edge of eyelid. This lump is in fact a small pimple which is appeared because of infection or inflammation in eyelash roots or oil glands within the eyelid. Touching eyes with contaminated hands (especially with nasal discharge) and inflammation in the edge of eyelid (blepharitis) are considered as some important factors causing sty. (7, 8, 9)

Rosa nutkana from Rose family (Rosaceae) is a shrubby plant, hibernal with small leaves and evergreen. It is fully resistant to coldness, heat and humidity. The species is locally native of northern hemisphere (California, Canada, Colombia, and Western North Pacific).

The height of the bush can reach up to 1.5 to 2 meters. The plant grows weedily on the coast of sloughs and flat surfaces in a pile. The calycles of the rose are located in the form of 3 to 5 into two rows. The calycles are in pinkish red color. The flowers are single and the end of

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them is swelled and the color is varying from purple to red. Light green leaflets are located under the calycles and the jagged shape of them has a different appearance. The plant flowers in summers. Usable parts are calycles and leaflets. When they are completely grown, they are getting collected by picking from the peduncle and spread in the form of slim layer. The species is native of northern hemisphere, but the other species are growing in Iran, (10, 11) have been collected from cold and humid areas.

According to scientific articles, antibacterial and antiviral features of Rosa nutkana and also consideration of substance-containing sepals (12), this family contains cyanogenic glycosides, saponin, tannin, oil, alcoholic carbohidrates, cyclytol, terpenoids and musilage. Alkaloids and saponin are rare in this family though.

Rose sepal contains a lot of components within which the most important ones are: trepens, acids, flavonoids, tannins and glycosides (13). A flavonoid named quercetin is involved in fennel combinations. From acid category we can refer to: tormentic acid, uscaphic acid, ursolic acid, malic acid and ascorbic acid. There are also catchin galat and glycoside combinations from tannin category in this extract (14). During world war II English scientists investigated that Rose fruits were a good source of vitamin C, so that three average red rose fruits could provide vitamin C as much as an orange. In northern and western parts of the country some jams and juices are made with Damascus Rose which is useful (e.g. as laxative) because of its minerals. Rose fruits contain 0.5 to 1.7 percent ascorbic acid, but vitamin C content of this product depends completely on herbal source, cultivation place, area climate, time and the way it is dried. In fact Red Rose fruits in the market lack vitamin C, even if ascorbic acid is high in Damascus Rose up to 1% and all of it is completely used in supplied products such as infused substances, soup and jam. Vitamin C increases body strength against illnesses and strengthens lungs, so it is useful for patients with weak bodies and low immunity. Rose-leaf tea prevents symptoms of common cold, influenza, coryza and bronchial spasm.

Different types of Rose prevent digestion system infections and their consumption after a period of antibiotic therapy results in balance of useful and harmless bacteria in digestion system. Rose leaves act as decongestant and diuretic, therefore they can disinfect poison and unwanted substances through producing more urine. Astringent components of Rose can stop bleeding, mucosa dryness and diarrhea. It is also useful for liver (15). Methanol extract has a restraining effect on MRSA.

In this study, some combinations such as tormentic acid, uscaphic acid, ursolic acid, quercetin, catechin, ascorbic acid and glycosides were found after analyzing the extract. The structure of each one was then determined using Mass Spectrometry method (13). Because of resistant staphylococcus in hospital environment and eye infections in Iranian patients, this study is carried out to evaluate the reaction of staphylococcus resistant to antibiotics especially methicillin, isolated from 60 patients suffering from sty, to Rosa nutkana extract.

Patients and Methods

This is a descriptive study and the research sample comprises 60 patients suffering from sty at remedial center of Shahid Beheshti University. Sampling was done from patients who had taken different antibiotics including methicillin and had no positive outcome. After sterile soap sampling, diagnostic tests were done and 19 cases of MRSA were reported. An ophthalmologist sampled patients by sterile soap. The sample was then put in technical sampling basis (TSB) environment and transferred to diagnostic microbiology lab at the university. It was incubated in 37 °C for 24 hours and then microscope slides were provided with gram staining done. After observation of gram-positive cocci, sample was transferred to blood agar environment and cultivated. After 24 – 48 hours in the situation mentioned, colonies were observed. Sampling and gram staining was repeated and the sample was transferred to monitor salt agar environment and DNase, passing catalase test which should be positive. Being certain about *staphylococcus aureus* existence, antibiotic test was done using McFarland, diffusion disc and agar dilution methods.

Complete sepal was first collected, then dried in cold and shade and grounded with a mill. Afterwards, 250 mg of ground plant was put in a 1000 cc flask and covered with 85% methanol dissolver. For extraction, each time about 600 ml methanol was poured on the powder in percolator and the solution was decanted. When 85% methanol dissolver was ready, it was poured on the powder until the powder was saturated and covered for about 2 cm. percolator door was closed and remained in the same position for 72 hours. Then the tap was opened gently and dissolved extract was gathered drop by drop. This was called primary extract.

To obtain secondary extract, methanol was added again and placed in the same position for 48 hours. Extraction was done in the same way. At the final stage, secondary extract was put in the same position for 24 hours and then extracted until a light extract was resulted showing that all the extract was obtained. This was done until the material became completely colorless. Then methanol decanted liquid was concentrated by rotary evaporator set. Pitch-like extracts were kept in a closed dark dish in a fridge and protected from light (16).

Extract concentration

After collection, extract was concentrated by rotary and some of its alcohol evaporated. Then the extract which was still liquid was poured in round-bottom flask (they break after connecting to the set). Extract was then changed into powder by lyophilization set. This set lowers the solution temperature until it freezes and changes into a solid porous mass. The mass was separated from flask by a glassy mixer.

Results

The comparison of antimicrobial effect of antibiotics with Rosa nutkana sepal extract showed that *staphylococcus aureus* was 100% resistant to amoxicillin while 100% sensitive to Rosa nutkana sepal extract. Moreover, this microorganism had 31.6% resistance, 52.6% intermediate sensitivity and 15.8% sensitivity to cephalothin.

Additionally, to tetracycline it showed 15.8% resistance, 57.9% intermediate sensitivity, and 26.3% sensitivity while to co-amoxiclav had 52.6% resistance, 15.8% intermediate sensitivity, and 31.6% sensitivity (diagram1).
According to this comparison, pseudomonas had 100% resistance to amoxicillin, cephalothin, tetracycline and co-amoxiclav while to Rosa nutkana sepal extract it had 100% sensitivity (diagram2).
The comparison implied that amoxicillin and co-amoxiclav had 100% resistance in Candida growth, but cephalothin showed 100% intermediate sensitivity and tetracycline and Rosa nutkana sepal extract 100% sensitivity (diagram3).

According to the results, the non-growth area of *staphylococcus aureus* was more in Rosa nutkana sepal extract than antibiotics used in this study. There was not any non-growth area for amoxicillin (diagram 4).
Moreover, the non-growth area of pseudomonas was more in Rosa nutkana sepal extract than antibiotics. There was not any area of non-growth in amoxicillin (diagram 5).
Furthermore, the area of non-growth was more in Rosa nutkana sepal extract than antibiotics and again there was not any area of non-growth in amoxicillin regarding MRSA (diagram 6).

Diagrams

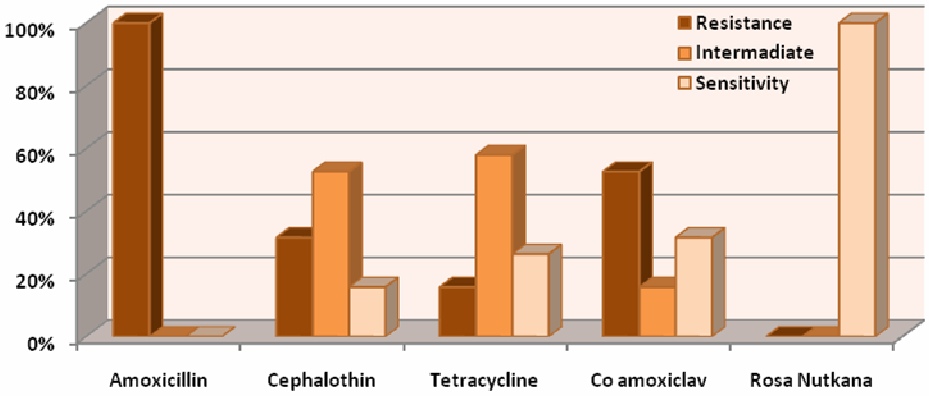

Diagram 4.1. Comparison of the effects of common antibiotic discs and Rosa nutkana sepal extract on *staphylococcus aureus* growth (in percent)

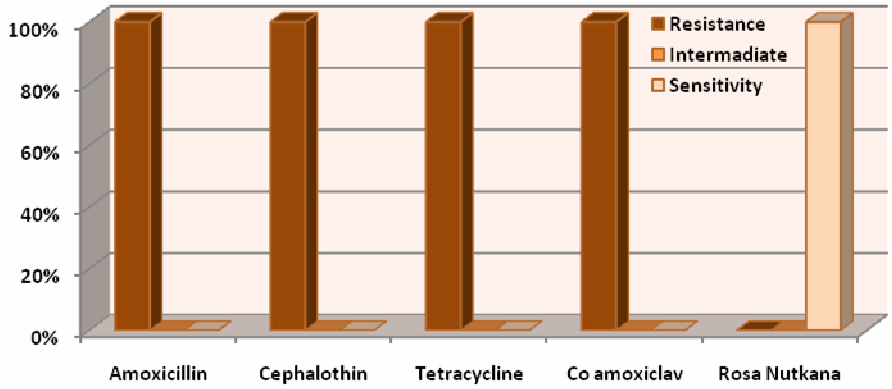

Diagram4.2. Comparison of the effects of common antibiotic discs and Rosa nutkana sepal extract on pseudomonas growth (in percent)

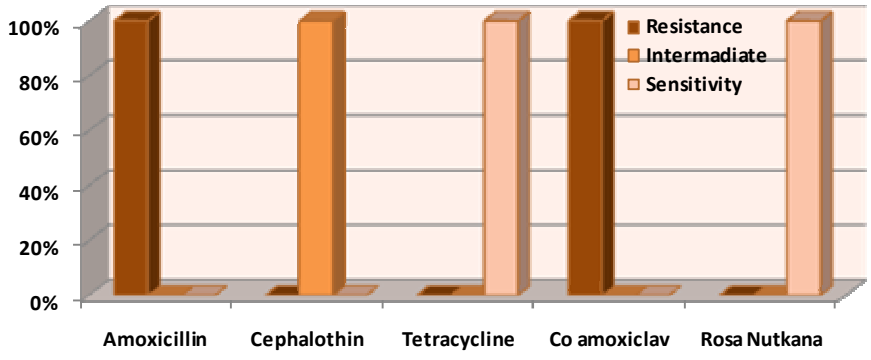

Diagram 4.3. Comparison of the effects of common antibiotic discs and Rosa nutkana sepal extract on aureus growth (in percent)

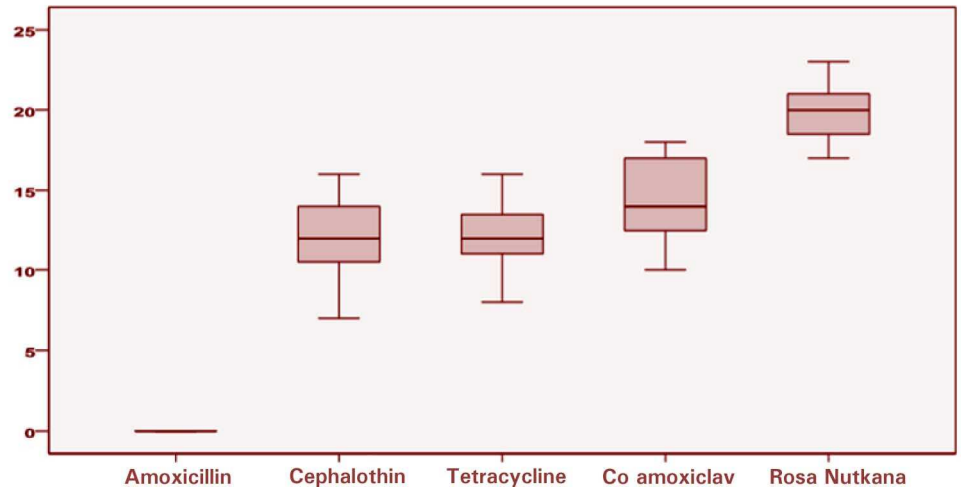

Diagram 4.4. Comparison of the effects of common antibiotic discs and Rosa nutkana sepal extract on *staphylococcus aureus* growth (in percent)

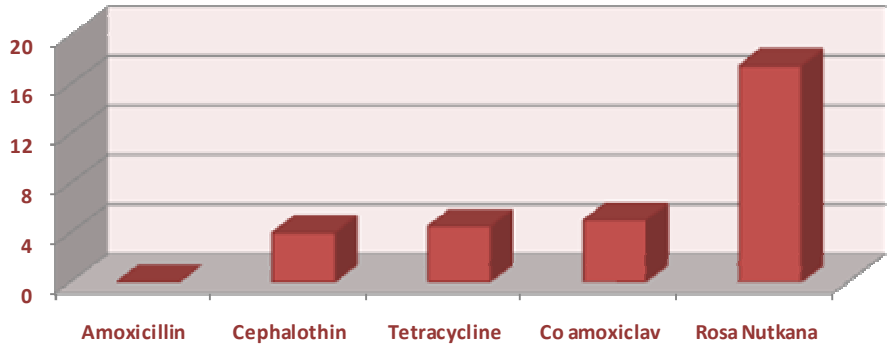

Diagram 4.5. Comparison of the effects of common antibiotic discs and Rosa nutkana sepal extract on pseudomonas growth (in percent)

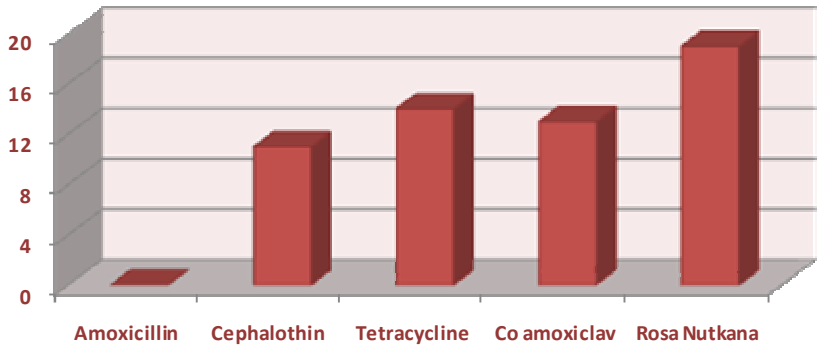

Diagram 4.6. Comparison of the effects of common antibiotic discs and Rosa nutkana sepal extract on *staphylococcus aureus* growth (in percent)

Discussion

Herbal products have a widespread popularity and ever-increasing consumption because of their natural source.

In this study the effect of Rosa nutkana sepal extract on MSRA was investigated. This bacterium is highly resistant and causes bacterial eye infections such as sty, and is also one of the biggest treatment problems. In a study on antibacterial effects of this plant, antibacterial effects against *staphylococcus aureus* was examined by agar dilution and disc diffusion methods. Rosa nutkana methanol extract had a great effect on MRSA, pseudomonas aeruginosa and *Candida albicans*, but there has been no research on antibacterial effect of sepal aqueous extract of this plant. This persuaded us to do the research. At first bacteria responsible for infection, medical sensitivity and resistance against routine antibiotics in sty were identified and then the results were compared with extract using antibiogram (disc diffusion) test and broth dilution method.

MSRA was sensitive to extract and among antibiotics, co-amoxiclav was reported with the highest effect. Therefore one of the probable reasons for observing no antibacterial effects in common synthetic antibiotics is that the effective substance includes antibacterial effects in tormentic acid extract or catechin gallate. Furthermore, MRSA was reported resistant to co-amoxiclav.

Another factor which may influence antibacterial effects of extract is extraction method and dissolver type.

Various extracts obtained by different methods and dissolvers can have different antibacterial effects on a special bacteria (17). Another influencing factor is cultivation environment for antibacterial experiments. The relation between cultivation environment and antibacterial effects of a substance has been proven (18).

Rosa nutkana extract has been introduced as a strong antimicrobial extract in previous studies. In a research done by Jovel and his colleagues methanol extract of this plant had a good effect on MRSA (19). Also YIO and his colleagues considered Rosa nutkana and investigated some features as antimicrobial, anti yeast and gram positive bacteria (20). Another study by McCutcheos identified the extract antivirus effects against para-influenza III, and corona virus (21). Furthermore, in a study on uscaphic acid isolated from Rosa nutkana extract by Chen and his colleagues some features such as anti inflammatory, antitussive, expectorant, and antidiabetic were found (22).

Average area of non-growth using Rosa nutkana sepal extract on *staphylococcus aureus* was 20 $\pm$ 2 mm and in cephalothin disc 12 $\pm$ 2.5 mm. Therefore, there was a meaningful difference between average of non-growth using Rosa nutkana sepal extract and cephalothin on staphylococcus aureus.

Area of non-growth in Rosa nutkana sepal extract was more than of antibiotics. There was not also any area of non-growth in Amoxicillin.

Comparing the least restraining effect (MIC) of Rosa nutkana sepal extract showed that with 1/128 density, it can influence staphylococcus aureus, pseudomonas, and Candida.

Conclusion

In conclusion, according to this study and because some bacteria causing sty were sensitive to Rosa nutkana sepal extract, it can be used as an antibiotic for management of this infection. With considering the obtained result in this study we conclude that Rosa nutkana can be suggested as co-treatment factor with ordinary antibiotics therapy.As most research shows MRSA is an important difficulties in micribiological therapy domain .combination therapy of Rosa nukana and antibiotics might have an important usage in sty treatment.

Recommendations

Regarding the effectiveness of the Rosa nutkana sepal extract against resistant bacteria in Sty infections, following recommendations are proposed:

1. In this study methanol extract has been used, so it is recommended to conduct researches on the extract obtained from other methods too.
2. In this study the antibacterial effect of the extract on Sty infections was investigated, so it is recommended to consider other effects of this extract too.
3. Considering the antibacterial effect of marigold extract on gram positive bacteria such as MRSA, it is recommended to study its effect on other gram positive bacterial infections and other diseases.

References

1. European pharmacopoeia 5th Ed.Main volume5.0, 2005 with supplements 5.1 and 5.2 (European pharmacopoeia)
2. Shahidi Bonjar G. H, Farrokhi, P. R. Anti- bacillus Activity Of Some Plants Used In Traditional Medicine Of Iran. Nigerian Journal of Natural Products and Medicine Vol.8, pp. 34-39, 2008.
3. Draelos Z.D (ed), Cosmetics in Dermatology, Noyes publications, USA, 1995, pp 214-220, 252.
4. Brooks George F. Jawetz,Mehnick and Adelberg's Medical Microbiology, 23th edition 2004
5. Mahhon C, Manuselis, G. Text book of Diagnostic Microbiology.2th 2000. pp. 364,473,475,477,487.
6. Shalaes D. M, Archer G. Antimicrobial resistance: new directions. ASM News 1991;57 :455-463.
7. Gawronska M, Kaluzny J, Mikucka A Gospodarek E. Bacteriological contants of conjunctival sac in patients with cataract in the early post operative period. Klin oczna 2005; 107(7-9) :414-7
8. Kasper D. L, Braunwald E and Fauci, A. S. Harrison's principles of internal medicine. Chapter 113, Introduction to Infectious Diseases: Host–Pathogen Interactions, McGraw-Hill, 2008.
9. External disease and cornea. Atlecht , EdD Hal Straus, Margaret Denny, Jack Daniel , Maxime Garrett. Academy Ophthalmology vol. cornea 2001
10. *Rosa nutkana*. 2007. Available from: http://www.rosa.com/conduct.html. Accessed Nov 25,2009.
11. *Nootka rose*.2006.Available from:http://www.wikipedia.org/rosa nutkana. Accessed June 30,2009.
12. Kaur T, Bijarnia R. K, Singla S. K. and Tandon C, Purification and characterization of an anticalcifying protein from the seeds of Trachyspermum ammi (L.). Protein and Peptide Letters, Vol. 16, No. 2, pp. 173-181, 2009
13. Mecutcheon AR,[et all].Antiviral screening of British Colombia medicinal plants.1995.Available from: http://www.pubmed.com.conduct.html. Accessed Jul 14, 2008.
14. Evans WC.*Trease andEvans pharmcogonsy*.14thFd.london:WB Sounders company Ltd;1996.p.250,329,226,230
15. Habibzadeh,Ali reza. *Pink Rose*. Available from: http://www.google.com/flower rose. accessed : jully 13.2009
16. Moschella SL, Hurley Hj. *Dermatology*. 3rd ed philodelphia pa: W.B. Saunders Co: .1992: 183, 223, 728-732.
17. Nostro A, Germano MP, Angelo VA, Marino A, Cannatelli MA, *Extraction methods and bio autography for evaluation of medicinal plant anti microbial activity*. Lett Appl Microbiol. 2000, 30 (5): 389-94.
18. Tassou CC, Drosinos EH, Nychas Gj. *Effect of essential oil from mint (Menta peperita) on salmonella entritidis and listeria monocttogenes in model food systems at 4 degrees and 10 degrees* C.j Appl Bacteriol. 1995 jun: 76(6): 593-600.
19. Jovel EM, Zhouxl, Mina DS, wahbe TR, Toweres GH. *Bioagtivity-guided isolation of the active compounds from Rosa nutkana and quantitive analysis of ascorbic acid by HPLC*. 2007 Available from:http//www.pubmed.com/archive.Accessed Jul 14, 2008.
20. Yio ,Jovel EM,Tower GH,Wahbe TR ,Cho D .*Antioxidant and antimicrobial activites of native Rosa sp.erom British Columbia, Canada*. 2007. Available from: http://www.pubmed.com.accessed Jul 14, 2008.
21. McCutcheon AR [et al].*Antiviral screening of British Columbia medicinal plants.* 1995. Available from: http://www.pubmed.com .Accessed Jull 14, 2008.
22. Chen J, Liw L, Wu JL, Ren BR, Zhang Ha. *Euscafiphic Acid a new hypoglycemic natural product from Follium Eriobofryae*. 2008. available from http://www.pubmed.com. Accessed nov 22,2009.