European Journal of Medicinal Plants
4(8): 937-945, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Analysis of Phytochemical and Antibacterial Activity of *Carissa spinarum* Linn Crude Extracts

Clarence Rubaka[1], Patrick Ndakidemi[1], Hamisi Malebo[2] and Francis Shahada[1*]

[1]School of Life Sciences and Bioengineering, The Nelson Mandela African Institution of Science and Technology, P.O. Box 447, Arusha, Tanzania.
[2]Department of Traditional Medicine, National Institute for Medical Research, P. O. Box 9653, Dar-es-Salaam, Tanzania.

Authors' contributions

This work was carried out in collaboration between all authors. Authors CR and FS designed the study, wrote the protocol, performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Authors CR and HM conducted phytochemical analysis and antibacterial activity. Authors FS, PN and HM were involved in supervising and guiding the progression of the study. All authors read and approved the final manuscript.

Original Research Article

Received 6th December 2013
Accepted 9th February 2014
Published 31st May 2014

ABSTRACT

Aims: To screen for the antibacterial activity of *Carissa spinarum* L. crude extracts against *Escherichia coli* and *Staphylococcus aureus*. The phytochemicals that are responsible for the bioactivity were also screened.
Study Design: *In vitro* assay of antibacterial properties.
Place and Duration of Study: Samples were collected from Samunge village at Loliondo in Ngorongoro district located in northern Tanzania. Extraction and phytochemical analyses were conducted at the Department of Traditional Medicine of the National Institute of Medical Research (NIMR) in Dar-es-Salaam, Tanzania. Antimicrobial bioassay was carried out at Department of Molecular Biology and Biotechnology at the University of Dar-es-Salaam between March 2013 and June 2013.
Methodology: Disk diffusion test was used to determine antimicrobial activity of the plant extracts. Chemical tests were used to determine the group of phytochemicals present in

**Corresponding author: Email: francis.shahada@nm-aist.ac.tz;*

the sample extracts.
Results: Sensitivity testing results indicated that *S. aureus* was found to be more sensitive than *E. coli*. *Carissa spinarum* L. methanolic extracts were the most active constituents and demonstrated the zone of inhibition values of 11.66±0.42 mm and 13.33±1.53 mm in diameter against *E. coli* and *S. aureus,* respectively. The highest percentage values of relative inhibition zone diameter of 57.24% (*E. coli*) and 70.17% (*S. aureus*) were demonstrated by *C. spinarum* L. root methanolic extracts. In contrast, *C. spinarum* L. bark extracts did not show any antibacterial activity against the two bacterial species. Plant extracts demonstrated the minimum inhibitory concentrations ranging from 312 to 5000 µg/ml. Phytochemical screening of crude extracts revealed the presence of saponins, alkaloids, flavonoids, tannins and sterols. The presence of these phytochemicals in the extracts was linked with observed antibacterial activity.
Conclusion: This study has revealed that the antibacterial activity of different extracts of *C. spinarum* L. was attributed to the presence of several phytochemicals. The study findings suggest likelihood of designing and developing potentially active antibacterial agents from *C. spinarum* L.

Keywords: Carissa spinarum L.; extracts; antibacterial activity; phytochemicals.

1. INTRODUCTION

Infectious diseases involving organisms such as bacteria, fungi, viruses and nematodes pose serious health problems worldwide and account for almost 50,000 deaths daily [1]. The situation has further been complicated with a dramatic increase of multidrug resistance by the pathogens with respect to the currently available classes of antimicrobials [2,3]. In the area where there is poor sanitation and ignorance of good hygienic practices, a large number of people are exposed to infectious agents. In addition, many developing countries are in tropical conditions which favor survival and multiplication of many disease causing agents and vectors posing a serious risk to the public health [4]. Plants have been used as a remedy for human diseases since antiquity because they contain components of therapeutic importance [5,6,7]. The World Health Organization has estimated that about 80% of the populations in the developing countries still rely on traditional medicines [8]. Developing new antimicrobials from different sources like natural products as alternative chemotherapeutic agents to antibiotics has been suggested to be one of the solutions against drug resistance [9]. Plant extracts can help in reducing drug resistance by providing an alternative mechanism which previously the pathogen were not adapted to resist [9,10]. Screening of the presence of antimicrobial compounds in medicinal plants helps to find the candidates which further can be developed into potential chemotherapeutic agents for the treatment of several diseases caused by microorganisms [11].

Carissa spinarum L. is a shrub found in family Apocyneceae widely distributed in tropical regions [12]. The plant may grow from 0.5-3 m with glossy green leaves and branches bearing thorns of 2-3 cm [13]. The plant also contains white shaped star flowers and ovate green berries which turn black or dark when ripe. In traditional system of medicine, the plant is used as a purgative, treatment of rheumatism and snake bites, and cleaning of worm-infested wounds of animals [13]. Some studies have investigated the presence of phytochemicals in *C. spinarum* and revealed the presence of cardiac glycosides, sesquiterpens, lignans and phenolic compounds in the stem [14,15]. The lignans extracted from the stem of *C. spinarum* Lin India demonstrated to have radical scavenging activity with 2, 2-diphenyl-1-picrylhydrazyl [14].

Due to the application of various parts of the plant in treating different ailments in traditional medicine, this study aims (i) to investigate the antibacterial activity of the root, leaf and bark extracts of *C. spinarum* Land (ii) to determine the phytochemical components of the plant.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

The root, leaf and bark samples of *C. spinarum* L. were collected from Samunge village located in Loliondo district in northern Tanzania. Collected plant materials were shade dried at room temperature before packed into containers and stored for extraction.

2.2 Preparation of Extracts

One hundred grams of powdered plant parts were soaked for 48 h with occasional shaking in 1.5 L of 95% ethanol, methanol and petroleum ether. After 48 h the extracts were filtered off by using Whatman number 1 filter paper (Scharlab, Barcelona, Spain) and the concentrate evaporated to dryness under reduced pressure and temperature in respect to the boiling point of a solvent (ethanol extracts at 78ºC, methanol at 65ºC, petroleum ether at 60ºC) by using rotary evaporator [16]. The concentrate extracts were stored at room temperature until used.

2.3 Standard Strains of Microorganisms Employed in the Study

The standard microorganisms used in this study were *Escherichia coli* DSM 1103 and *Staphylococcus aureus* ATCC 25923. The microbes were obtained from the Department of Molecular Biology and Biotechnology at the University of Dar-es-Salaam, Tanzania. The microbes were seeded in the respective plates and incubated at 37ºC for 24 h. After incubation, the colonies were picked by using sterilized loop, transferred to the tubes containing nutrient broth, and incubated at 37ºC for 4-6 h. The bacterial concentration was adjusted to 0.5 McFarland standards.

2.4 Antimicrobial Susceptibility Testing

Standard test bacteria (*E. coli* and *S. aureus*) were inoculated onto the surface of sterile solidified MHA plates and sterile glass spreader was used for even distribution of the inoculums as previously described [17]. Sterile filter paper discs (4 mm in diameter) were soaked in the extract solutions each at the concentration of 10 mg of extract in 1ml of 5% DMSO. The discs were placed on different MHA plates inoculated with different test organisms. Then, the plates were left for 1 h to allow diffusion of extracts and antibiotics. After 1 h the plates were incubated at 37ºC for 24 h. The filter paper disc soaked in DMSO was used as a negative control and the discs containing ciprofloxacin (15 µg) and ampicillin (30 µg) were used as positive controls. Antibacterial activity was determined by measuring the inhibition zone diameter (mm) against each test organism. The antimicrobial activity expressed as percentage relative inhibition zone diameter (RIZD) was calculated as previously described [9]; % RIZD = (IZD sample – IZD negative control) × 100/ IZD antibiotic standard where; RIZD is the percentage of relative inhibition zone diameter and IZD is the inhibition zone diameter (mm).

2.5. Measurement of the Minimum Inhibition Concentration

The minimum inhibition concentration (MIC) of individual plant extracts were determined according to the method previously described [18] with modification. Two-fold serial dilutions of the compounds were made with nutrient broth. Extract stock solutions (10mg/ml) were serially diluted in seven test tubes to the concentrations of 10, 5, 2.5, 1.25, 0.625, 0.3125 and 0.156 mg/ml. The antibiotic stock concentration was serially diluted to 1, 0.5, 0.25, 0.125, 0.0625, 0.03125 and 0.0156 mg/ml. The tubes were pipetted with 1ml of the microbial suspension and incubated at 37ºC for 24 h. The control tubes did not have any antibiotic or test compound included, but contained the test bacteria and the solvent used to dissolve the extracts. After incubation, the visual turbidity was observed and recorded. The lowest concentration in which the turbidity was not observed was measured as an MIC of the specific extract.

2.6 Phytochemical Tests

Determination of the presence of alkaloids, flavonoids, tannins, terpenoids and sterols was done qualitatively using the protocols previously described [19,20,21].

2.7 Statistical Analysis

The statistical analysis was performed using analysis of variance (ANOVA), with the computations being performed with the software program CoStat. The fisher's least significance difference (L.S.D.) was used to compare treatment means at p = 0.05 level of significance.

3. RESULTS

3.1 Inhibitory Effects of Extracts against *S. aureus* and *E. coli* Strains

The inhibitory effects of *C. spinarum* L. extracts against *S. aureus* and *E. coli* were determined. The results of zone of inhibition (ZOI) and percentage relative inhibition zone diameter (RIZD) against tested pathogens are presented in Table 1. The extracts demonstrated significant different ($P \leq 0.05$) in antibacterial activity against *S. aureus* with zone of inhibition ranging from zero to 13.33±1.00 mm. Percentage of relative inhibition zone diameter exhibited by plant extracts against *S. aureus* ranged from zero to 70.13%. *Carissa spinarum* L. root methanolic extracts demonstrated the highest value of the antibacterial activity (13.33±1.53mm) against *S. aureus*. In contrast, *C. spinarum* L. bark ethanolic extracts did not show any activity against the bacteria. The highest percentage value of RIZD (70.13%) was demonstrated by *C. spinarum* L. root methanolic extract against *S. aureus* when ciprofloxacin was used as a standard antibiotic. Besides, 66.97% RIZD was observed when ampicillin was used as the standard antibiotic.

The extracts demonstrated significant difference ($P \leq 0.05$) in antibacterial activity against *E. coli* with zones of inhibition ranging from zero to 11.66±0.47 mm. Percentage of relative inhibition zone diameter exhibited by plant extracts against *E. coli* ranged from zero to 66.97% (Table 1). *Carissa spinarum* L. root methanolic extract demonstrated the highest value (11.66±0.42 mm) of the antibacterial activity. In contrast, *C. spinarum* L. bark extract did not show any antibacterial activity. The highest percentage value of RIZD (57.24%) was demonstrated by *C. spinarum* L. root methanolic extract against *E. coli* when ciprofloxacin

was used as the standard antibiotic while 54.66% RIZD was observed when ampicillin was used as the standard antibiotic.

Table 1. Inhibition zone diameter of individual ethanolic, petroleum ether and methanolic plant extract and antibiotics

Extract/ Drug		Zone of inhibition (mm)		Percentage RIZD			
	Concentration of extract (mg/ml) and standard antibiotic (µg/disc)	*S. aureus*	*E. coli*	*S. aureus*		*E. coli*	
		ZOI	ZOI	1	2	3	4
CSLP	10	8. 66±1.53c	6.66±2.51c	48.08	45.92	43.28	41.32
CSRE	10	10.00±1.00c	9.33±1.53b	51.91	49.57	51.25	48.94
CSRM	10	13.33±1.53b	11.66±0.47b	70.13	66.97	57.24	54.66
CSBE	10	0.00±0.00d	0.00 ±0.00d	0	0	0	0
CSLM	10	8.33±1.53c	2.33±0.58d	46.53	44.43	13.73	13.11
AMP	30	20.66±1.53a	19.66±2.08a	100	100	100	100
CIP	15	19.66±1.53a	19.00±1.00a	100	100	100	100
DMSO		0	0	0	0	0	0

CSLP, C. spinarum L. leaves petroleum ether extract; CSRE, C. spinarum L root ethanolic extract; CSRM, C. spinarum L root methanolic extract; CSBE, C. spinarum L bark ethanolic extract; CSLM, C. spinarum L leaves methanolic extract; AMP, ampicillin and CIP, ciprofloxacin; RIZD, relative inhibition zone diameter; ZOI, zone of inhibition. 1 and 2 indicate percentage RIZD when ciprofloxacin was used as a positive control; 3 and 4 indicate percentage RIZD when ampicillin was used as the positive control.Value of zone of inhibition of the extracts reported as the mean of the three replicates ± standard deviation (SD). Means followed by dissimilar letter in a column are significantly different from each other at P = 0.05 according to Fischer's Least Significant Difference (LSD).

3.2 Phytochemical Analysis of Crude Extracts

Phytochemical screening of crude extracts of *C. spinarum* L. revealed the presence of bioactive components as shown in Table 2. The present study revealed *C. spinarum* L. leaves methanolic extract containing high amount of saponins compare to others parts tested as it developed a strong persistent foam lasting over 16 h period. Besides, the extracts contained sterols, tannins and flavonoids. Conversely, it was found that *C. spinarum* L. extracts did not contain any alkaloid.

Table 2. Phytochemical analysis of individual ethanolic, petroleum ether and methanolic plant extracts

Phytochemical	CSLM	CSLP	CSRM	CSRE	CSBE
Saponins	++	+	+	+	+
Alkaloids	-	-	-	-	-
Tannins	+	+	+	+	+
Flavonoids	+	+	+	+	+
Sterols	+	+	+	+	+

CSLM, C. spinarum L leave methanolic extracts; CSLP, Carissa spinarum L leaves petroleum ether extract; CSRM, C. spinarum L root methanolic extract; CSRE, C. spinarum L root ethanolic extract and CSBE, C. spinarum L bark ethanolic extract.+, present; ++, abundantly present and -, absent.

3.3 Minimum Inhibition Concentration

Plant extracts demonstrated MICs ranging from 312 to 5000 µg/ml (Table 3). *Carissa spinarum* L. leaves petroleum extract and *C. spinarum* L. root methanolic extract displayed the lowest (312 µg/ml) MIC value against *S. aureus* while *C. spinarum* L. root ethanolic extract and *C. spinarum* L. root methanolic extract demonstrated the lowest (312 µg/ml) MIC value against *E. coli*.

Table 3. Minimum inhibition concentration (µg/ml) of *Carissa spinarum* L. crude extracts

Extract/Drug	Microorganism	
	S. aureus	***E. coli***
CSLP	312	2500
CSRE	1250	312
CSBE	5000	5000
CSLM	1250	2500
CSRM	312	312
AMP	32	32

CSLP, Carissa spinarum L. leaves petroleum ether extract; CSRE, C. spinarum L root ethanolic extract; CSBE, C. spinarum L bark ethanolic extract; CSLM, Carissa spinarum L leave methanolic extract; CSRM, C. spinarum L root methanolic extract and AMP, ampicillin.

4. DISCUSSION

The results observed in this study indicate that the extracts of *C. spinarum* L demonstrated antibacterial activity against the two tested pathogens, *S. aureus* a Gram-positive and *E. coli* a Gram-negative bacteria. The plant extracts demonstrated lower antibacterial activity against *E. coli* when compared to *S. aureus*. The reason which makes *S. aureus* to be more susceptible to antibacterial agent than *E. coli* might be attributed to their structural differences [22]. Gram-negative bacteria contain an outer phospholipid membrane with the structural polysaccharide components which make their cell wall impenetrable to antimicrobial agent while Gram-positive bacteria are more susceptible, having only outer peptidoglycan which is not an effective permeability barrier [23]. Nevertheless, another study on antibacterial activity of methanolic root extracts from *Carica papaya* showed that Gram-negative bacteria were more susceptible to antibacterial agent than Gram-positive bacteria, and recommended that the disparity of their results was attributed to the nature of the medium used that affected diffusability of the agent [2].

The value of zone of inhibition exhibited by *C. spinarum* L. root methanolic extract (13.33±1.53 mm and 11.66±0.47 mm against *S. aureus* and *E. coli*, respectively) is a good indication of the highest efficacy against these bacteria. Different levels of antibacterial activity observed in the present study were attributed to the presence of phytochemicals screened. The presences of bioactive compounds in medicinal plants have been linked with antimicrobial properties of crude plant extracts [5,6,7,24]. The screening of phytochemicals in *C. spinarum* L. revealed the presence of tannins, alkaloids, saponins, sterols and flavonoids. Tannins such as tannic acid and propyl gallate inhibit growth of food-borne bacteria [25]. Tannins have the ability to inactivate microbial adhesins, enzymes, cell envelope, transport proteins and polysaccharides [25]. The flavonoids isolated from the leaves of *Ocimum sanctum* have shown to exhibit antibacterial activity against *S. aureus*, *Staphylococus cohni*, *E. coli*, *Proteus* and *Klebsialla pneumonia* [26]. The hydroxyl group of

flavonoids attributes to antioxidant and chelating action to enhance antimicrobial activity [6,27]. Saponin, the natural detergent, has the ability of forming stable foam in water [28]. The present study showed *C. spinarum* L. leaf methanolic extract containing more saponin than other extracts, hence, linked with exhibited antibacterial activity. Antimicrobial activity of saponin is associated with the ability of forming pore in the cell membrane and hence giving the toxic material free access to the cell [28]. Alkaloids have been credited as good source of many drugs and destruct the microbe by intercalating the DNA [25]. However, results of the present study showed alkaloids were absent in *C. spinarum* L. extracts. Additionally, results of phytochemical screening in the present study showed the presence of sterols in all plant extracts. The sterols extracted from difference parts of *Euphorbia hirta* have demonstrated antibacterial activity against different pathogens including *S. aureus, Bacillus subtilis*, *Proteus mirabilis*, *Raoultella planticola*, *E. coli, Enterobacter aerogens* and *Pseudomonas aeruginosa* [29].

The findings of the present study also indicate that plant extracts inhibited growth of the pathogens at different MIC values. The plant extracts with high activity against a particular organism usually gives low MIC value while the extracts with low activity gives high MIC value [2]. The present study demonstrated *C. spinarum* L. leaf petroleum ether extract and *C. spinarum* L. root methanolic extract have higher activity than other extracts when tested against *S. aureus*. Besides, *C. spinarum* L. root ethanolic and methanolic extracts demonstrated high activity when tested against *E. coli*. Bioactive compounds in the roots of *C. spinarum* L. have wide spectrum of antibacterial activity showed by low MIC values in both *E. coli* and *S. aureus* bacteria.

Ampicillin and ciprofloxacin, which were used as positive controls and at the lower concentration, produced larger zones of inhibition against tested pathogens than the plant extract tested. This could be explained by the fact that the extracts were still in their crude form made up of complex composition of chemicals compared to the standard drugs which were pure compounds. The suggestion might explain as to why there was lacking of activity observed from *C. spinarum* L. bark ethanolic extract. Further purification of the extracts could lead to isolation of pure compounds with increased antimicrobial activity or application of the combination therapy amongst the extracts to potentiate their activity [16].

5. CONCLUSION

This study has revealed the antibacterial activity of different extract of *C. spinarum* L. attributed by the presence of several phytochemicals. The study suggests likelihood of future designing and development of potentially active antibacterial agents from *C. spinarum* L. for the treatment of infectious diseases. Further phytochemical and pharmacological studies are needed to isolate the active constituents and evaluate their antimicrobial activity against a wide range of pathogens.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTEREST

Authors have declared that no competing interests exist.

REFERENCES

1. Idowu AO, Oluremi BB, Seidu NI. Incidence and susceptibility pattern of clinical isolates from pus producing infection to antibiotics and *Carica papaya* seed extract. Afr J Biotechnol. 2011;10(9):1700-1704.
2. Doughari JH, Elmahmood AM, Manzara S . Studies on the antibacterial activity of root extracts of *Carica papaya L.* Afr J Microbiol Res. 2007;37-41.
3. Sibanda T, Okoh A. The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr J Biotechnol. 2007;6(25).
4. World health organization. World health organization report on infectious diseases. WHO/CDS/99.1;1999.
5. Betoni JE, Mantovani RP, Barbosa LN, Di Stasi LC, Junior ANF. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. Memórias do Instituto Oswaldo Cruz, Rio de Janeiro. 2006;101:387-390.
6. Cowan, MM. Plant products as antimicrobial agents. Clin Microbiol Rev. 1999;12:564–582.
7. Lalitha V, Kiran B, Raveesha KA. *In vitro* evaluation of Mimusops. Elengi L. Plant extracts for antibacterial activity and phytochemical analysis. Pharmacophore. 2011;2(1):78-85.
8. World health organization. Promoting the role of traditional medicine in health systems. A strategy for the African region. AFR/RC50/9; 2000.
9. Rojas JJ,Veronica JO, Saul AO, John FM. Screening for antimicrobial activity of ten medicinal plants used in Colombian folkloric medicine: A possible alternative in the treatment of non-nosocomial infections. BMC Comp Alt Med. 2006;6(1):2.
10. Stapleton PD, Shah S, Taylor PW. Potentiation of catechin gallate-mediated sensitization of *Staphylococcus aureus* to oxacillin by non galloylated catechins. Int J Antimicrob Agents. 2006;23(5):462-467.
11. Bohlin L, Bruhn JG, editors. Bioassay method in natural product research and drug development. Klumar. Academic Publisher. Netherlands; 1999.
12. Sanwal R, Chaudhary AK. Wound healing and antimicrobial potential of *Carissa spinarum* Linn in albino mice. J Ethnopharmacol. 2011;135:792-796
13. Fatima A, Singh PP, Agarwae P, Irchhaiya R, Alok S, Amita V. Treatment of various diseases by *Carissa spinarum L.*A promising shrub. Int J Pharm Sc Res. 2013;4(7):2489-2495.
14. Rao RJ, Kumar US, Reddy SV, Tiwari AK, Rao JM. Antioxidants and a new germacrane sesquiterpenes from *Carissa spinarum*. Nat Prod Res. 2005;19:763–769.
15. Wangteerraprasert R, Lipipun V, Gunaratnam M, Neidle S, Gibbons S, Likhitwitayawuid K. Bioactive compounds from *Carissa spinarum*. Phytother Res. 2012;26:1496-1499.
16. Akinyemi AI, Ogundare AO. Antibacterial properties of the leaf extract of *Anthocleista djalonensis* A. chev on some pathogenic organisms. Eur J Med Plants. 2013;4(1):75-85.
17. Doughari JH, Ndakidemi PA, Human IS, Benade S. Antioxidant, antimicrobial and Antiverotoxic potentials of extracts of *Curtisia dentata*. J Ethnopharmacol. 2012;141:1040-1050.

18. Chung PY, Navaratnam, Chung LY. Synergistic antimicrobial activity between pentacyclic triterpenoids and antibiotics against *Staphylococcus aureus* strains. Ann Clin Microbiol Antimicrob. 2011;10(25).
19. Mamta S, Jyoti S. Phytochemical screening of *Acorus calamus* and *Lantana camara.* Int Res J Pharm. 2012;3(5).
20. Sindhu CG. Phytochemical screening of *Calendula officinalis* Linn leaf extract by TLC. Int J Res Ayurveda Pharm. 2010;(1):131-134
21. Doherty VF, Olaniran OO, Kanife UC. Antimicrobial activity of *Aframomum melegueta* (Allegator pepper). Int J Biol. 2010;2(2).
22. Nikaido H, Vaara M. Molecular basis of bacterial outer membrane permeability. Microbiol Rev. 1985;1:1-32.
23. Firas A, Al-Bayati. Isolation and identification of antimicrobial compound from *Mertha longifolia L.* leaves grown in Iraq. Ann Clin Microbiol Antimicrob. 2009;8:20.
24. Sharma C, Aneja KR, Kasera R. Screening of *Berberis aristata* DC. For antimicrobial potential against the pathogens causing ear infections. Int J Pharmacol. 2011;7(4):536-541
25. Akiyama H, Fujii K, Yamasaki O, Oono T, Lwatsuki K. Antibacterial action of several tannins against *Staphylococcus aureus*. J Antimicrob Chemother. 2001;48(4):487-491.
26. Ali H, Dixit S. *In vitro* antimicrobial activity of flavonoids of *Ocimum sanctum* with synergistic effect of their combined form. Asian Pac J Trop Dis. 2012;396-398.
27. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nut Biochem. 2002;13(10):572-584.
28. Abid MM, Khan A, Naqvi ST, Nagvi SM. Identification of phytosaponins as novel biodynamic agents. Asian J Biol Sci. 2012;3(3):459-467.
29. Singh G, Kumar P, Jindal A. Antibacterial potential of sterols of some medicinal plants. Int. J Pharm Pharm Sci. 2012;4(3).

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=519&id=13&aid=4757