British Microbiology Research Journal
8(4): 532-539, 2015, Article no.BMRJ.2015.145
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antimicrobial Properties of *Ocimum sanctum and Calotropis gigantea* Leaves

Carrell Sarah Bansavatar[1], Rajini Kurup[2*] and Abdullah Adil Ansari[1]

[1]Faculty of Natural Sciences, University of Guyana, Turkeyen Campus, Guyana.
[2]Faculty of Health Sciences, University of Guyana, Turkeyen Campus, Guyana.

Authors' contributions

This work was carried out in collaboration between all authors. Author CSB designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors AAA and RK performed the statistical analysis, managed literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/17062
Editor(s):
(1) Lingan Rajendran, Tamil Nadu Agricultural University, India.
Reviewers:
(1) Shadia El Rafie, Chemical Engineering Department, National Research Centre, Egypt.
(2) Anonymous, Iuliu Hatieganu" University of Medicine and Pharmacy Cluj-Napoca, Romania.
(3) Anonymous, Pt. JNM Medical College, India.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=1090&id=8&aid=9260

Original Research Article

Received 25th February 2015
Accepted 29th April 2015
Published 14th May 2015

ABSTRACT

Objective: To evaluate antimicrobial and phytochemical activity of hexane and ethanolic extract of *Ocimum sanctum* and *Calotropis gigantea leaves* on clinical pathogens.
Materials and Methods: The agar diffusion test was used to check the antimicrobial activity of the hexane and ethanolic extracts of the medicinal plants. Three different concentrations of the tested agents were used for the study. The values of Zone of Inhibition were tabulated according to the concentration of the tested agent and data was statistically analyzed using ANOVA tests. Phytochemicals were also detected in all the four extracts.
Results: All the plants extracts showed considerable antimicrobial activity against selected clinical pathogens. Hexane and ethanolic extracts of *Ocimum sanctum* and *Calotropic gigantea* leaves showed strong antibacterial activity against *Staphylococcus aureus* at the 4% concentration. At 4% concentration the ethanolic extract of *Ocimum sanctum* has a greater effect on the tested microbes, having a mean and standard deviation of 8.37±0.57 on the most resistant microbe; being *Escherichia coli*. Phytochemical analysis of active extracts demonstrated the presence of common phyto-constituents like tannins, glycosides, saponins, flavonoids and alkaloids.
Conclusion: The hexane and ethanolic extract of *O. sanctum* and *C. gigantea* has considerable antimicrobial activity against *S. aureus, E. coli and Klebsiella* spp.

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

Keywords: Antimicrobial; traditional medicine; phyto-constituents; disc diffusion.

1. INTRODUCTION

The predominant cause of global mortality and morbidity is lifestyle related diseases which can be addressed through Ayurveda with its focus on healthy lifestyle practices and regular consumption of adaptogenic herbs [1]. *O. sanctum* (tulsi) is an aromatic shrub in the basil family *Lamiaceae* that is thought to have originated in north central India and now grows throughout eastern world tropics [2]. It is also believed that daily consumption of *O. sanctum* could prevent disease, promote general health and treat conditions like anxiety, cough, asthma, diarrhea, fever, dysentery, arthritis, indigestion, vomiting, genitourinary disorders, back pain, skin diseases, insect, snake and scorpion bites [3-7].

O. sanctum is also known to prevent cancers caused by toxic compounds by reducing DNA damage and inducing apoptosis in precancerous and cancerous cells, thereby reducing the growth of experimental tumors and enhancing survival [8-10]. Studies has not only revealed anti-microbial activity of *O. sanctum* but also revealed that it boost defenses by enhancing immune responses in humans and animals [11-18]. *O. sanctum* has made an important contribution to the modern research due to its large number of medicinal properties like antibacterial antioxidant, anti-inflammatory and analgesic activities useful for wound healing. It has proven broad spectrum activity against *Streptococcus mutans* and treatment of bad breath, gum disease and mouth ulcers [19-24]. *O. sanctum* is also known to have antibacterial activity against normal tap water and river water human pathogen at a minimum concentration of 600 mg1-1 [25].

Another medicinal plant tested in this study was *C. gigantea* leaves, which is believed to cure several illnesses such as toothache, earache, sprain, anxiety, pain, epilepsy, diarrhoea and mental disorders since tribal ages. *C. gigantea* is scientifically reported for its anti-*Candida* activity, cytotoxic activity, antipyretic activity and wound healing activity [26-30]. Very few studies have been reported regarding the antibacterial activity of leaves of *O. sanctum* and *C. gigantea.*

The main objective of this research was to screen and evaluate the antimicrobial activity of extracts from the leaves of *O. sanctum* and *C. gigantea*; two plants used traditionally for their medicinal value by the people of Guyana, against bacterial pathogens responsible for prevalent infections in Guyana and other South American countries.

2. MATERIALS AND METHODOLOGY

2.1 Plant Collection

The leaves of *O. sanctum* and *C. gigantea* were collected from the Mahaica-Mahaicony, Guyana, authenticated by scientific officer at the Centre for the study of biological diversity herbarium located within the University of Guyana, Turkyen campus. Plant materials were washed with distilled water and air dried for one week, pressed for an additional week via the use of a plant press and mounted on acid free cardboard using standard herbarium preservation technique. All the materials were ground in an electric mill and stored in tight light-protected containers.

2.2 Extraction of Phytochemicals

Cold percolation method was used to extract the various phyto-constituents from the leaves of the different plants. Approximately three hundred grams (300 g) of the individual plant powder was weighed using an electronic balance and their individual phyto-constituents were then extracted using the cold percolation method. This method involved the soaking of the grind leaves in glass jars for forty eight hours (48 hrs). For this experiment two solvents extractions were redistilled and used by placing the solvents individually into a distiller apparatus which separates mixtures based on different volatilities, pure hexane was redistilled at a boiling point of 69°C while 95% ethanol was redistilled at a boiling point of 78.1°C, the solvents were collected and stored in air tight-glass containers.

2.3 Identification Test for Phytochemicals (Secondary Metabolites)

Tests were done to detect the presence of Flavonoids, Tannins, Reducing sugars, Alkaloids, Glycosides, Terpenoid and steroid. These test for the various phytochemicals were done for both hexane and ethanolic extracts of *O. sanctum* and *C. gigantea* leaves.

2.4 Formulation of the Different Concentrations for Each Test Extract

Different concentrations were made using the stock solutions of hexane and ethanolic extracts

of the leaves of *O. sanctum* and *C. gigantea* (0.1%, 1% and 4% concentrations).

2.5 Formula Used in the Calculations for Acquiring the Different Concentration

Formula=$C_1V_1=C_2V_2$

$C_{1=}$ initial concentration; $V_{1=}$ initial volume; $C_{2=}$final concentration; $V_{2=}$ final volume

2.6 Test Microorganisms

The microbial strains were obtained from clinical isolates from Georgetown Public Hospital Cooperation, Guyana. The bacterial strains tested were *Staphylococcus aureus*, *Escherichia coli,* and *Klebsiella spp.* Microorganisms were maintained at 10°C on nutrient agar plates.

2.7 Antimicrobial Susceptibility Test: Disc Diffusion Method

All test organisms were inoculated in Mueller Hinton broth (pH 7.4.) for 8 hours. The concentration of the suspensions was adjusted to 0.5 McFarland standards. Isolates were spread on Mueller Hinton agar plates by using sterilized cotton swabs.

The previously prepared discs of concentrations 0.1%, 1%, 4% of hexane and ethanolic extract of *O. sanctum* and *C. gigantea* were arranged radially on the agar plate. Tetracycline antibiotic disc was used as positive control and sterilized distilled water disk was used negative control. Plates were incubated at 37°C for 48 hours. Triplicate plates were maintained for each organism. Zone of inhibition were measured with transparent ruler in millimeter.

2.8 Statistical Analysis

The collected data was analyzed using the following statistical test (SPSS 20)

1. Mean value and standard deviation
2. One-way analysis of variance

3. RESULTS

Phytochemical analysis of hexane and ethanolic extracts of *O. sanctum* and *C. gigantea* indicated that all extracts tested negative for flavones and only ethanolic extract of the leaves of *O. sanctum* testing positive for alkaloids. Glycosides, Steriods and Terpenoids were present all four samples (Table 1).

The antimicrobial activities of the extracts of *C. gigantea* and *O. sanctum* leaves are listed in Table 2. All values were expressed as mean±standard deviation of three replicates. The results were statistically analyzed by student t test at P≤0.05 confidence limit. The ethanol and hexane extract of *C. gigantea* and *O. sanctum* leaves exhibited antibacterial activity against three clinical isolates of bacteria (Figs. 1 and 2). Extract showed maximum antibacterial activity against all the three pathogen at 4% concentration of *O. sanctum* ethanol extract. It is observed that there is a low standard deviation for all the diameter of zones of inhibition this implies that the data obtained from the triplicates of the antimicrobial susceptibility test did not deviate much from the mean. Zone of inhibition against *C. gigantea* Ethanol and O. sanctum Hexane are shown in the Figs. 3 & 4.

The Anova test greater F value than F crit value, therefore there is significant differences between the different concentrations of all the four extract treatment on colonies of *S. aureus, E. coli* and *Klebsiella* spp. which indicates that the extract is statistically and clinically effective in inhibiting the growth of these bacteria. On the other hand, the different concentrations of the sample extract have a similar effect on the different bacteria colonies with no statistical significant difference (F value <Fcrit) (Table 3).

Table 1. Phytochemicals analysis of all the tested samples

Phytochemicals	*C. gigantea* hexane	*C. gigantea* ethanol	*O. sanctum* hexane	*O. sanctum* ethanol
Flavonoid	+	+	-	+
Flavones	-	-	-	-
Gallic tannins	+	+	-	+
Catecholic tannins	-	-	+	+
Glycosides	+	+	+	+
Steriods	+	+	+	+
Terpenoids	+	+	+	+
Alkaloids	-	-	-	+

There were significant differences of the between different concentrations of the tested samples on the three microbes. In each case the statistical data obtained showed that the F-calculated value was always greater than the F-critical value at 0.05 probability level. Hence, the different dilutions of tested samples had significantly different effect on the size of inhibition zone (Table 4).

4. DISCUSSION

Plant extracts have been used for thousands of years, in pharmaceuticals, alternative medicine and natural therapies. The beneficial effect of plant materials typically results from many naturally occurring compounds found in plants known as secondary metabolites; it is usually not attributed to a single compound but a combination of the metabolites [31]. The healing properties of medicinal plants and herbs are well accepted since ancestral period and scientifically very well proven today [32]. Even in the western countries, like United States, a variety of herbal therapies has been used [33,34].

In vitro studies in this work showed that both *O. sanctum* and *C. gigantea* leaves extracts inhibited bacterial growth; however their effectiveness at different concentrations varied. The results of this study were in agreement with previous studies where different concentrations of Tulsi have been used against all three tested microorganisms [35-39]. The biological properties of the plant has been attributed to the presence of active compounds like Ursolic acid, flavonoids (epigenin, orientin and vicenin) [40] and phenolic compounds (cirsilineol, circimaritin, isothymusin, eugenol) [41]. The antibacterial activities tulsi has proved against gram positive and gram negative bacteria including enteric organisms [42-44]. Similar to this study, methanolic extracts and aqueous suspension of leaves of tulsi showed immmunomodulatory properties with secretion of cytokines. Studies of tulsi have shown a good analgesic, antipyretic as well as anti- inflammatory activities [45,46].

Aqueous extract of *C. gigantea* showed high inhibitory activity followed by methanol extract, whereas ethanol and petroleum ether extracts showed low activity [47]. Previous studies report the presence of phytochemicals like cardenolides, flavonoids, terpenes, pregnanes, nonprotein amino acid and cardiac glycoside as major constituents in *C. gigantea* may acknowledge the medicinal property of this plant [48,49]. *C. gigantea* extracts have shown significant antimicrobial effect against endodontic pathogens especially against *S. mutans* and also against *S. aureus* and *E. faecalis* at higher concentration [50-54]. In support with this study *C. gigantea* leave extracts showed high inhibitory activity with aqueous extract followed by methanol extract against different species of Candida. At lower concentration ethanol extract showed good inhibitory activity against clinical isolates [55,56].

Table 2. Values of zone of Inhibition at different concentrations of tested agents

Concentration (%)	*C. gigantea* hexane	*C. gigantea* ethanol	*O. sanctum* hexane	*O. sanctum* ethanol
S. aureus				
0.1	0±0	5.60±0.360	5.37±0.25	6.03±0.15
1	5.77±0.06	6.97±0.76	6.07±0.21	7.63±0.55
4	6.93±0.70	7.73±0.58	6.9±0.36	9.2±0.26
E. coli				
0.1	0±0	1.77±3.06	0±0	1.93±3.35
1	5.23±0.15	6.2±0.82	5.73±0.29	6.83±0.76
4	6.57±0.58	7.3±0.61	6.43±0.42	8.37±0.57
***Klebsiella* spp.**				
0.1	0±0	3.8±3.31	0±0	1.97±3.401
1	5.53±0.21	6.60±0.78	5.83±0.32	7.20±0.70
4	6.67±0.58	7.47±0.57	6.67±0.29	8.77±0.42

Table 3. ANOVA analysis: Two way factor analysis

ANOVA: Two factor without replication			
***O. sanctum* hexane**	**F**	**P value**	**F crit**
Rows	7.2	0.0	6.9
Columns	1.4	0.3	6.9
***O. sanctum* Ethanol**			
Rows	17.8	0.0	6.9
Columns	2.4	0.2	6.9
***C. gigantea* hexane**			
Rows	1928.0	0.0	6.9
Columns	3.4	0.1	6.9
***C. gigantea* ethanol**			
Rows	13.3	0.0	6.9
Columns	2.4	0.2	6.9

Table 4. Anova: Two way analysis

ANOVA: Two factor without replication			
S. aureus	**F**	**P-value**	**F crit**
Rows	6.5	0.0	5.1
Columns	3.3	0.1	4.8
E. coli			
Rows	400.3	0.0	5.1
Columns	19.8	0.0	4.8
***Klebsiella* spp.**			
Rows	55.2	0.0	5.1
Columns	4.7	0.1	4.8

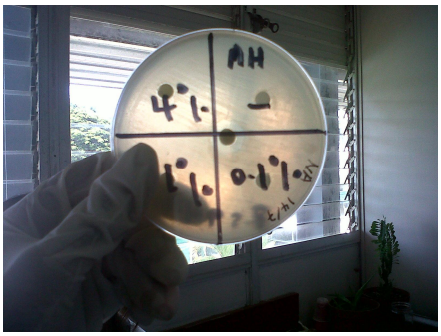

Fig. 1. Antimicrobial property of *C. gigantea* hexane

Fig. 3. Antimicrobial property of *C. gigantea* ethanol

Fig. 2. Antimicrobial property of *O. sanctum* ethanol

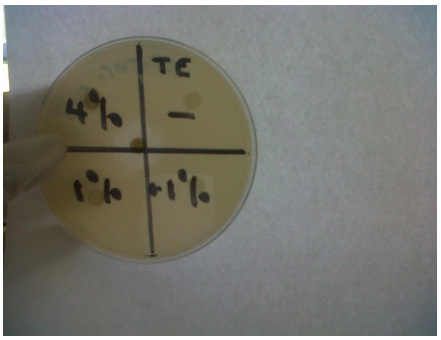

Fig. 4. Antimicrobial property of *O. sanctum* hexane

4. CONCLUSION

In conclusion, the results of the present study support the traditional usage of the *O. sanctum* and *C. gigantea*. This antibacterial study of these plant extracts demonstrated that traditional medicine can be as effective as modern medicine with an increase in their concentrations and this can lead to the combating of pathogenic microorganisms. The millenarian use these plants in folk medicine suggests that they represent an economical and safe alternative to treat infectious diseases.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Cohen MM. Tulsi – *Ocimum sanctum*: A herb for all reasons. J Ayurveda Integr Med. 2014;5(4):251-259.
2. Bast F, Rani P, Meena D. Chloroplast DNA phylogeography of Holy Basil (*Ocimum tenuiflorum*) in Indian subcontinent. Scientific World Journal. 2014;47482. DOI: 10.1155/2014/847482.
3. Singh N, Hoette Y, Miller R. Tulsi. The mother medicine of Nature. 2nd ed. Lucknow: International Institute of Herbal Medicine. 2010;28-47.
4. Mahajan N, Rawal S, Verma M, Poddar M, Alok S. A phyto-pharmacological overview in Ocimum species with special emphasis on *Ocimum sanctum*. Biomed Prev Nutr. 2013;3:185-92.
5. Mohan L, Amberkar MV, Kumari M. *Ocimum sanctum* linn. (TULSI) – an overview. Int J Pharm Sci Rev Res. 2011; 7:51-3.
6. Pattanayak P, Behera P, Das D, Panda SK. *Ocimum sanctum* Linn. A reservoir plant for therapeutic applications: An overview. Pharmacogn Rev. 2010;4:95-105.
7. Mondal S, Mirdha BR, Mahapatra SC. The science behind sacredness of Tulsi (*Ocimum sanctum* Linn.). Indian J Physiol Pharmacol. 2009;53(4):291-306.
8. Siddique YH, Ara G, Beg T, Afzal M. Anti-genotoxic effect of *Ocimum sanctum* extract against cyproterone acetate induced genotoxic damage in cultured mammalian cells. Acta Biologica Hungarica. 2007;58(4):397–409. DOI: 10.1556/abiol.58.2007.4.7
9. Jha AK, Jha M, Kaur J. Ethanolic extracts of *Ocimum sanctum*, *Azadirachta indica* and with aniasomnifera cause apoptosis in SiHa cells. Res J Pharm Biol Chem. 2012; 3:557-62.
10. Manikandan P, VidjayaLetchoumy P, Prathiba D, Nagini S. Combinatorial chemo preventive effect of *Azadirachta indica* and *Ocimum sanctum* on oxidant-antioxidant status, cell proliferation, apoptosis and angiogenesis in a rat fore stomach carcinogenesis model. Singapore Med J. 2008;49(10):814.
11. Vasudevan P, Kashyap S, Sharma S. Bioactive botanicals from basil (*Ocimum* sp.) J Sci Ind Res (C). 1999;58:332-8.
12. Mediratta PK, Sharma KK, Singh S. Evaluation of immune-modulatory potential of *Ocimum sanctum* seed oil and its possible mechanism of action. Ethno Pharmacol. 2002;80(1):15-20.
13. Hemalatha R, Babu KN, Karthik M, Ramesh R, Kumar BD, Kumar PU. Immuno-modulatory activity and Th1/Th2 cytokine response of *Ocimum sanctum* in myelo suppressed swiss albino mice. Trends Med Res. 2011;6:23-31.
14. Tripathi AK, Rajora VS, Gupta DK, Shukla SK. Immuno-modulatory activity of *Ocimum sanctum* and its influence on cyclophosphamide induced immunosuppression. Indian J Anim Sci. 2008;78:33-6.
15. Goel A, Singh DK, Kumar S, Bhatia AK. Immuno-modulating property of *Ocimum sanctum* by regulating the IL 2 production and its mRNA expression using rat's splenocytes. Asian Pac J trop Med. 2010;3:8-12.
16. Pacaraj M, Balasubramanian V, Baskaran S, Ramasamy P. Development of immunity by extract of medicinal plant *Ocimum sanctum* on common carp *Cyprinu scarpio* (L.) Res. J Immunol. 2011;4:12-8.
17. Chitra G, Krishnaveni N. Immuno-stimulatory effect of *Ocimum sanctum* leaf extract on the Indian major carp, Catla catla. Plant Archives. 2011;11:213-4.
18. Manikandan P, Mondal S, Varma S, Bamola VD, Naik SN, Mirdha BR et al. Double-blinded randomized controlled trial for immune-modulatory effects of Tulsi (*Ocimum sanctum* Linn.) leaf extract on healthy volunteers. J Ethnopharmacol. 2011;14:136(3):452-6. DOI: 10.1016/j.jep.2011.05.012.

Epub 2011 May 17.
19. Kukrejab J, Dodwad V. Herbal mouthwashes – A gift of nature. Int J Pharma Bio Sci. 2012;3:46-52.
20. Malik K, Arora G, Singh I. *Ocimum sanctum* seeds, a natural super disintegrant: Formulation and evaluation of fast melt tablets of nimesulide. Polim Med. 2012;42:49-59.
21. Agrawal P, Nagesh L. Comparative evaluation of efficacy of 0.2% Chlorhexidine, Listerine and Tulsi extract mouth rinses on salivary *Streptococcus mutans* count of high school children-RCT. Contemp Clin Trials. 2011;32:802-8.
22. Malhotra, R, Grover V, Kapoor A, Saxena D. Comparison of the effectiveness of a commercially available herbal mouth rinse with chlorhexidine gluconate at the clinical and patient level. J Indian Soc Periodontol. 2011;15:349-52.
23. Singh S, Taneja M, Majumdar DK. Biological activities of *Ocimum sanctum* L. fixed oil-an overview. Indian J Exp Biol. 2007;45:403-12.
24. Shetty S, Udupa S, Udupa L. Evaluation of antioxidant and wound healing effects of alcoholic and aqueous extract of *Ocimum sanctum* Linn in rats. Evid Based Complementary Altern Med. 2008;5:95-101.
25. Kayastha BL, Queen of herbs tulsi (*Ocimum sanctum*) removes impurities from water and plays disinfectant role. J Med Plants Stud. 2014;2(2). ISSN: 2320-3862.
26. Gaurav Kumar, Karthik L, Bhaskara Rao KV. *In vitro* anti-Candida activity of *Calotropis gigantea* against clinical isolates of Candida, Journal of Pharmacy Research. 2010;3:539-542.
27. Wang Z, Wang M, Mei W, Han Z, Dai H. A New Cytotoxic pregnanone from *Calotropis gigantean.* Molecules. 2008;13:3033-3039.
28. Chitme HR, Chandra R, Kaushik S, Evaluation of antipyretic activity of *Calotropis gigantean* (Asclepiadaceae) in experimental animals. Phytother Res. 2005;19:454-456.
29. Saratha V, Subramanian S, Sivakumar S, Evaluation of wound healing potential of *Calotropis gigantean* latex studied on excision wounds in experimental animals. Med Chem Res; 2009.
DOI: 10.1007/s00044-009-9240-6.
30. Kumar G, Karthik L, Venkata Bhaskara Rao. Antibacterial activity of aqueous extract of *Calotropis gigantea* leaves – an *In vitro* study. Inter J Pharm Sc Rev. 2010;4(2).
31. Joshi B, Lekhak S and Sharma A. Antibacterial property of different medicinal plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana*. Kathmandu University Journal of Science, Engineering and Technology. 2009;5:143-150.
32. Sharma AK, Kumar A, Yadav SK, Rahal A. Studies on Antimicrobial and Immunomodulatory effects of hot aqueous extract of *Acacia nilotica* L. leaves against common veterinary pathogens. Vet. Med. International. 2014;747042.
DOI: 10.1155/2014/747042.
33. Tiwari R, Latchumikanthan A, Yadav RK, Dhama R. Herbal and traditional botanical medicines for safeguarding animal and human health. Livestock Line. 2012;6:29-33.
34. Rahal A, Mahima, Verma AK, Kumar A, Tiwari R, Kapoor S, Chakraborty S, Dhama K. Phytonutrients and nutraceuticals in vegetables and their multi-dimensional medicinal and health benefits for humans and their companion Animals: A Review. J Biolo Sci. 2013;14(1):1-19.
35. Agarwal P, Nagesh L, Murlikrishnan. Evaluation of the antimicrobial activity of various concentrations of Tulsi (*Ocimum sanctum*) extract against *Streptococcus mutans*: An *In vitro* study. Indian J Dent Res. 2010;21:357-9.
36. Mishra P, Mishra S. Study of antibacterial activity of *Ocimum sanctum* extract against gram-positive and gram-negative bacteria. Am J Food Technol. 2011;6:336-41.
37. Geeta, Vasudevan DM, Kedlaya R, Deepa S, Ballal M. Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against the enteric pathogens. Indian J Med Sci. 2001;55:434-8,472.
38. Sharma A, Chandraker S, Patel VK, Ramteke P. Antibacterial activity of medicinal plants against pathogens causing complicated urinary tract infections. Indian J Pharm Sci. 2009;71: 136-9.
39. Joshi B, Sah GB, Basnet BB, Bhatt M, Sharma D, Subedi K, Pandey J, Malla R. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia*

caryophyllata (clove), *Achytanthes Bidentata* (Datiwan) and *Azadirachta Indica* (Neem). J Microbiol Antimicrob. 2011;3:1-7.
40. Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. As a medicinal plant. Indian J Exp Biol. 2002;40:765-73.
41. Indian Herbal Pharmacopoeia. Mumbai, India: IDMA. 2002;272.
42. Gaurav Kumar, Karthik L, Bhaskara Rao KV. *In vitro* anti-Candida activity of *Calotropis gigantean* against clinical isolates of Candida. J Pharm Res. 2010; 539-542.
43. Wang Z, Wang M, Mei W, Han Z, Dai H. A New Cytotoxic Pregnanone from *Calotropis gigantean.* Molecules. 2008; 3033-3039.
44. Ali M, Gupta J, New penta cyclictriterpenic esters from the roots of *Calotropis procera.* Indian J Chem. 1999;877-881.
45. Bhatia AK, Kumar A, Goel A, Gupta A, Rahal A. Antibacterial activity of hot Aqueous extract of *Ocimum sanctum* leaves against common bacterial pathogens of animals. Pharma Sci. Monit. 2013;4:279-285.
46. Kumar A, Rahal A, Verma AK. *In-vitro* antibacterial activity of hot aqueous extract (HAE) of *Ocimum sanctum* (Tulsi) leaves. Ind. J. Vet. Med. 2011;31:96-97.
47. Kumar A, Rahal A, Chakraborty S, Tiwari R, Latheef SK, Dhama K. *Ocimum sanctum* (Tulsi): A miracle herb and boon to medical science-A review. Int. J. Agron. Plant Prod. 2013;4:1580-1589.
48. Bhatia AK, Kumar A, Goel A, Rahal A, Immunomodulatory activity of hot aqueous extract of *Ocimum sanctum* leaves. Indian J. Compa. Microbiol. Immunol. Infect. Dis. 2013;34: 33-37.
49. Kumar A, Rahal A, Chakraborty S, Tiwari R, Latheef SK, Dhama K, *Ocimum sanctum* (Tulsi): A miracle herb and boon to medical science-A review. Int. J. Agron. Plant Prod. 2013;4:1580-1589.
50. Mishra P, Mishra S. Study of antibacterial activity of *Ocimum Sanctum* extract against gram-positive and gram-negative bacteria. Am J Food Technol. 2011;6:336–41.
51. Geeta, Vasudevan DM, Kedlaya R, Deepa S, Ballal M. Activity of *Ocimum sanctum* (the traditional Indian medicinal plant) against the enteric pathogens. Indian J Med Sci. 2001;55: 434–8,472.
52. Sharma A, Chandraker S, Patel VK, Ramteke P. Antibacterial activity of medicinal plants against pathogens causing complicated urinary tract infections. Indian J Pharm Sci. 2009;71: 136–9.
53. Joshi B, Sah GP, Basnet BB, Bhatt M, Sharma D, Subedi K, Pandey J, Malla R. Phytochemical extraction and antimicrobial properties of different medicinal plants: *Ocimum sanctum* (Tulsi), *Eugenia caryophyllata (clove), Achyranthes bidentata (Datiwan)* and *Azadirachta indica (Neem).* J Microbiol Antimicrob. 2011;3:1–7.
54. Gupta SK, Prakash J, Srivastava S. Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn as a medicinal plant. Indian J Exp Biol. 2002;40: 765–73.
55. Kumar G, Karthik L, Bhaskara Rao KV. *In vitro* anti-Candida activity of Calotropis gigantea against clinical isolates of Candida. J Pharm Res. 2010;3:539-542.
56. Kumar G, Karthik L, Rao KVB. Antibacterial activity of aqueous extract of *Calotropis gigantea* leaves – an in vitro study. Int J Pharm Sci Rev Res. 2010; 4(2):024. ISSN 0976-044X.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=1090&id=8&aid=9260