British Microbiology Research Journal
10(1): 1-8, 2015, Article no.BMRJ.20002
ISSN: 2231-0886

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antibacterial Activity of Flavonoids Extracted from Seeds of *Pongamia pinnata* Linn on Methicillin Resistant *Staphylococcus aureus*

Mary Shobha Rani Inala[1], C. D. Dayanand[2*], Nagarjuna Sivaraj[1], P. M. Beena[3] and A. V. M. Kutty[3]

[1]Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.
[2]Department of Biochemistry/Head Allied Health Sciences, Sri Devaraj Urs Medical College, Kolar, Karnataka, India.
[3]Department of Microbiology, Sri Devaraj Urs Medical College, Kolar, Karnataka, India.

Authors' contributions

This work was carried out in collaboration between all authors. Authors CDD and PMB designed the study. Author NS performed the statistical analysis. Authors CDD and AVMK wrote the protocol and wrote the first draft of the manuscript and author MSRI managed literature searches. Authors MSRI and NS managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/20002
Editor(s):
(1) Marcin Lukaszewicz, Department of Biotransformation, Faculty of Biotechnology, University of Wroclaw, Wroclaw, Poland and Division of Chemistry and Technology Fuels, Wroclaw University of Technology, Wroclaw, Poland.
Reviewers:
(1) Anonymous, National University of San Luis, Argentina.
(2) Anonymous, Cumhuriyet University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/10465

Original Research Article

Received 6th July 2015
Accepted 23rd July 2015
Published 9th August 2015

ABSTRACT

Aims: To assess the antibacterial property of seed crude extracts of *Pongamia pinnata* Linn and isolated flavonoids component from crude extract against Methicillin resistant *Staphylococcus aureus* obtained from clinical isolates.
Study Design: Observational study.
Place and Duration of the Study: Department of Allied health sciences, Department of Biochemistry and Department of Microbiology in Sri Devaraj Urs Academy Of Higher Education and Research ,Tamaka, Kolar, between February 2014 and march 2015.

**Corresponding author: E-mail: cd8905@yahoo.co.in;*

Methodology: Confirmed clinical isolates for MRSA were collected from Microbiology department to test the efficacy of crude extracts of seeds from *Pongamia pinnata* L. Methanolic crude extract has been preferably used for isolation of flavonoid content using Dimethyl Sulfoxide [DMSO] and methanol as ideal solvents during extraction process by column chromatography technique. Agar well diffusion method was performed to determine the antibacterial activity of crude seed extracts of *Pongamia pinnata* and isolated flavonoids by using quercitin as positive control for flavonoids. Vancomycin a glycopeptide powder used as gold standard for comparing bactericidal activity of quercitin, flavonoids and crude extracts of *P. pinnata* on MRSA.
Results: The highest antibacterial activity (75-89%) was observed in crude extract of *Pongamia pinnata* in comparison to vancomycin considered as cent percent. Extracted flavonoids showed activity (66-92%) with respect to crude extract and (50-84%) with vancomycin and the activity (71-92%) with respect to quercitin when tested with concentration ranging from 25-400 µg/ml.
Conclusion: This study showed that seed extracts of *Pongamia Pinnata L* and its phytochemical compound flavonoids showed potential antibacterial activity against MRSA using quercitin and vancomycin.

Keywords: Agar well Diffusion; Pongamia pinnata Linn; flavonoid; vancomycin; quercitin; MRSA.

1. INTRODUCTION

Methicillin Resistant *Staphylococcus aureus* (MRSA) enhanced prevalence was reported in recent years [1]. Although vancomycin a glycopeptide antibiotic commonly used as drug of choice for MRSA infections, emergence of vancomycin resistant MRSA strain has become more rampant that alarms as serious global concern. This has made researchers to search for suitable therapeutic active agents from plants sources against various organisms [2,3] and also on MRSA organism detected in hospital environment [4].

In the present study, an attempt has been made to evaluate antibacterial activity from *Pongamia pinnata* L seed extract and its phytochemical content such as flavonoids action to determine the antibacterial effect on MRSA obtained from clinical isolates which are confirmed by the department of Microbiology.

The detection of MRSA infection is life threatening and has very few therapeutic options. This has led to screening of several medicinal plants from numerous families to evaluate the potential antimicrobial activity [5,6]. In our earlier study, the bactericidal effect of *Pongamia pinnata Linn* on various organisms isolated from the clinical isolates showed the potential antibacterial action on *S. aureus* [7]. This observation generated an idea to design a study and find out the similar effects on MRSA.

Pongamia pinnata L is a medium sized glabrous tree grows in South eastern Asia and Australia. In the Indian Literature, various parts of this plant have been suggested as medicine for various ailments [8]. The seeds of this plant are used for treating different inflammatory and infectious diseases such as leprosy, lumbago, leucoderma, muscular, and articular rheumatism [9-11].

However, there is very few information available on phytochemical compounds isolated from *Pongamia pinnata* seed extract showing antibacterial activity against MRSA. Therefore this study was undertaken to study flavonoid component as potential antibacterial agent against MRSA in comparison with known flavonoid quercitin

2. MATERIALS AND METHODS

2.1 Collection of *Pongamia pinnata* Linn Plant Seeds

Pongamia Pinnata Linn seeds were collected and authenticated from the Horticulture College, Tamaka, and Kolar. The collected seeds were de-shelled and good quality seeds were dried and used for our investigation.

2.2 Preparation of *P. pinnata* Seed Extract

The extraction of *Pongamia pinnata* seed was carried out by standard procedure [12]. The seeds were dried in shade and powdered in a mechanical grinder. Five grams of seed powder was initially defatted with petroleum ether for three–four times and further extracted with 50 ml of absolute methanol and extract was subjected for filtration by using whatman no1 filter paper.

The filtrate was concentrated in vacuum evaporator under reduced pressure and air dried. Thus obtained powder was stored in sterile bottles at 4°C until further use.

2.3 Collection and Laboratory Detection of MRSA from Clinical Sample by Cefoxitin disk Diffusion Method [13]

MRSA contains *mec*A gene responsible for resistance to betalactum antibiotics in addition to molecular genetic analysis. Cefoxitin (*mecA* gene inducer) disk diffusion test is the most accurate test for detection of presence of *mecA* gene in S aureus. The test procedure involves incubation of lawn of test isolates on Mueller Hinton agar and 2% sodium chloride under standardized conditions with a disc containing thirty microgram of Cefoxitin which is according to the Clinical and Laboratory Standards Institute (CLSI) that indicated a zone of inhibition around the Cefoxitin disk of ≥22 mm rules out MRSA. However, a zone size equal or less than 22 mm indicates the presence of *mecA* gene that confirms presence of MRSA in clinical isolate

2.4 Phytochemical Screening of *Pongamia pinnata* Seeds

Phytochemicals from the seed extract of *Pongamia pinnata* were qualitatively identified by using standard procedures [14,15] such as alkaloids, flavonoids, saponins, steroids, glycosides and tannins.

2.5 Isolation and Purification of Flavonoids from *P. pinnata* Seed Extract by Using Adsorption Column Chromatography

A glass column measuring 50 cm X 2 cms dimension developed using methanol with silica gel adsorbent on glass wool and allowed to settle by gravity flow. Column was allowed to equilibrate with suitable methanol as elution solvent. The even surface of the silica gel in the column protected by placing whatman filter paper disc 1 gm/ml of processed crude extract was applied for separation. All the eluted fractions were tested for flavonoid content, the active fractions were pooled and air dried under sterile conditions. The concentrated dried powder subjected for qualitative confirmation of flavonoids using Dimethyl sulfoxide as a dissolving solvent and quercitin as internal standard [16].

2.6 Qualitative Detection of Flavonoids [17]

Two ml of above extract was treated with few drops of 20% sodium hydroxide which produced intense yellow color, and on further addition of dilute hydrochloric acid becomes colorless confirming the presence of flavonoids.

2.7 Antibacterial Assay by Agar Well Diffusion Method

MRSA isolates grown on nutrient agar plates, was inoculated into nutrient broth and incubated for 4-6 h at 37°C to obtain organisms in log phase of growth. Broth turbidity was adjusted to 0.5 McFarland units to get 108 organisms /ml. These standard inoculums were inoculated on to Muller Hinton agar plates with a sterile glass spreader to distribute the inoculums equally. Wells were punched in the agar plate with help of sterile cork-borer.

The different concentration of P.pinnata crude extract and flavonoids, quercitin a commercially obtained flavonoid and vancomycin a gold standard was prepared using Dimethyl sulfoxide solvent. 20 µl from five different concentrations like 25 µg, 50 µg, 100 µg, 200 µg and 400 µg of crude extract, flavonoid, quercitin and vancomycin were added into respective agar plates inoculated with MRSA using a sterile pipette. Agar well with DMSO serves as internal control where as quercitin and vancomycin serves as positive controls. Then all the plates were incubated at 37°C for 48 hours. The antibacterial activity was assessed by measuring the zone of inhibition around the well .The experiments were performed in triplicates.

3. RESULTS

The experimental results obtained from the present study when tested using 25 to 400 µg/ml crude extract, flavonoids, quercitin and vancomycin indicated antimicrobial potential of *Pongamia pinnata* seeds on MRSA organism presented in Table 1.

Prominent antibacterial activity 89% observed in crude extract with a diameter of inhibitory zone of 24 mm when compared to standard antibiotic vancomycin powder (100%) that showed 27 mm inhibitory zone. The structure of vancomycin and quercitin is shown in Fig. 3.

The isolated and partially purified flavonoid content from the crude extract evinced 20 mm diameter inhibitory zone that amounts to 84% of inhibition with respect to vancomycin. However the flavonoid component on comparison with a known flavonoid member quercitin indicated 92% of inhibition at 400 µg/ml respectively as shown in Figs. 1&2.

The antimicrobial activity by flavonoids is similar with the study of Rudi hendra et al. [18].

The effect of flavonoid component on MRSA is on agreement with few research report on determination of antimicrobial activity by plants [19-23]. Generally quercitin is the widely distributed flavonoid from the flavonoid group and hence maximum antimicrobial activity exhibited by flavonoids of *Pongamia pinnata* confers the chief component would be the quercitin in flavonoid compounds isolated and tested. Similarly, crude extract showed 54% of inhibition with 15mm diameter of zone of inhibition when compared to vancomycin. In the same way 25 µg/ml flavonoids showed 66% of inhibition with 10 mm of zone of inhibition respectively.

Thus, the sensitivity and specificity of crude extract ranges from 25-400 µg/ml revealed antibacterial activity of 75-89% in comparison to vancomycin. Flavonoids in the same concentration range showed activity of 66-92%with respect to crude extract, 71-92% with respect to quercitin and 50-84% with vancomycin is shown in the Graph 1.

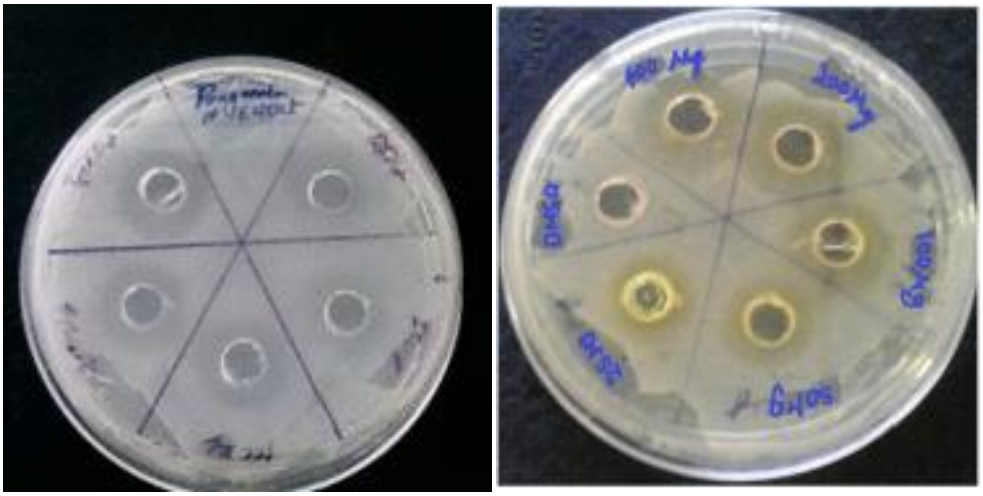


Fig. 1. Antibacterial activity of *Pongamia pinnata* crude extract and flavonoid against MRSA

Fig. 2. Antibacterial activity of vancomycin and quercitin against MRSA

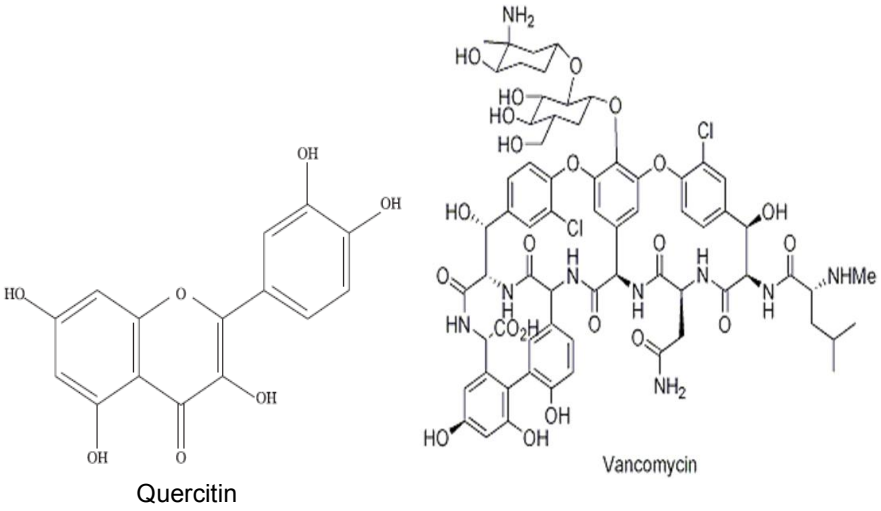
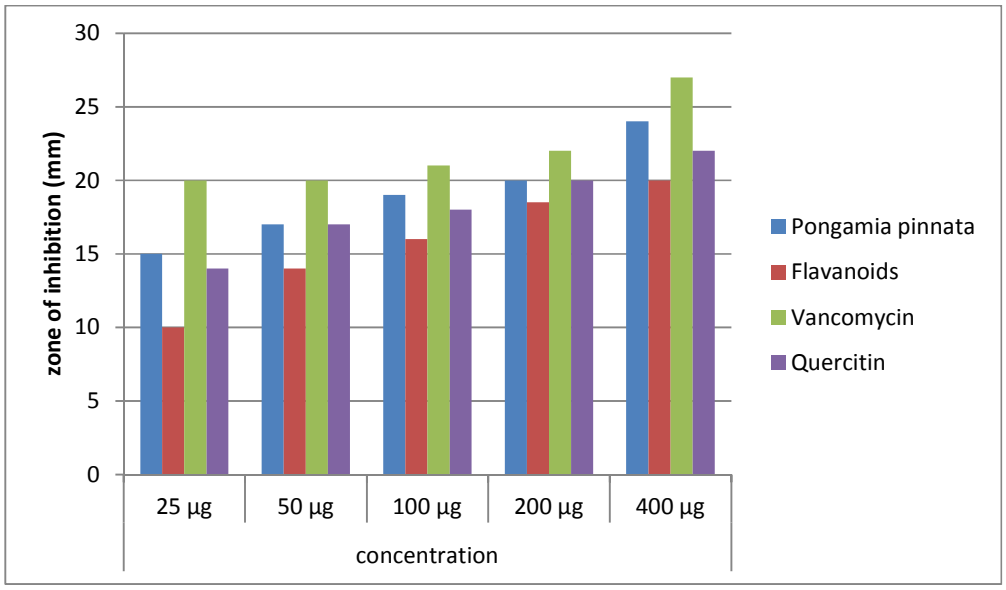


Fig. 3. Structure of querecitin and vancomycins

Table 1. Zone of inhibiton in mm at different concentrations against MRSA

Components	Zone of inhibition (mm)				
	Concentrations (µg/ml)				
	25	**50**	**100**	**200**	**400**
P. pinnata crude extract	15	17	19	20	24
Flavonoids	10	14	16	18	20
Quercitin	14	17	18	20	22
Vancomycin	20	20	21	22	27



Graph 1. MRSA inhibiton by crude, flavanoids of *P. pinnata* l with quercetin and vancomycin powder at diferent concentration

4. DISCUSSION

In a report of our previous study, the crude extract of *Pongamia pinnata L* seeds was tested on the pathogenic organisms of clinical isolates such as *Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Serratia marcescens, Micrococcus luteus* in comparison with antibiotic ceftizidime. The results obtained facilitate to evaluate bactericidal action of P. pinnata on MRSA organism in clinical isolates. An evidence for antibacterial property of flower extract of *Pongamia pinnata* against pathogens causing serious infections reported [24].

The phytochemical investigation of *Pongamia pinnata L* indicated the presences of abundant prenylated flavonoids such as furano flavoids, chromeno flavones [25,26].

Pongamia pinnata seed extract evidenced the presence of phytochemical compounds such as alkaloids, tannins, saponins, steroids, glycosides and flavonoids by using qualitative tests. However antibacterial activity was demonstrated by isolation of flavonoid component in the present study. There are studies which indicated antimicrobial activity by phytochemical constituent from various medicinal plants for their content namely phenolics, alkaloids, steroids, tannins, saponins etc. The qualitative results obtained are in agreement with other research reports.

Different phytochemicals were tested on antibacterial activity and found useful during infectious diseases [27]. Flavonoids is widely distributed in edible plants with less toxicity therefore have been used in therapeutic applications [28,29]. Flavonoids are ubiquitous in nature distributed in fruit, vegetables, nuts, stems, flower, seeds etc., they were isolated, purified and characterized to identify antifungal, antiviral and antibacterial activity.

Similarly in current study, Different concentration ranging from 25-400 µg /ml of crude seed extract and partially isolated and purified flavonoid component from crude seed extract demonstrated to evaluate bactericidal action on MRSA bacterium isolated and confirmed from clinical samples. The positive controls used were quercitin and vancomycin powder.

Limitation of the present study is that, apart from flavonoids, antibacterial activity was not carried out using other constituents to prove whether or not other components have antimicrobial activity on MRSA. Thereby any other compounds of seed extract may accounts for inhibition on MRSA. It is interesting to note that, isolated flavonoids when compared with a known flavonoid quercitin along with vancomycin powder against MRSA showed arbitrarily expected to nearest inhibitory effect indicates the presence of quercitin in this isolated flavonoids. Since, research work is ongoing in the institute and hence evaluation of antimicrobial activity with other bioactive compound(s) is to be tested, and the isolation and purification of quercitin from flavonoid extract and the mechanism of action of quercitin to be explored.

5. CONCLUSION

The study has proved that seed extracts of *Pongamia Pinnata L* and its phytochemical compound flavonoids showed potential antibacterial activity against MRSA using quercitin and vancomycin. Therefore, flavonoids have received much attention in the literature for their potential beneficial effect in anti-infective research.

ACKNOWLEDGEMENTS

We would like to thank the authorities of Sri Devaraj Urs Academy of Higher Education and Research, Department of Microbiology, Horticulture College Kolar for supporting this study. The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of methicillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet. 2006;368:874-85.
2. Farnsworth NR. Ethno pharmacology and future drug development: The North American experience. J Ethnopharmacol. 1993;38:145–52.
3. Houghton PJ. The role of plants in traditional medicine and current therapy. J Alter Complement Med. 1995;1:131-43.

4. Palombo EA, Semple SJ. Antibacterial activity of Australian plant extracts against methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). J Basic Microbiol. 2002;42(6):444-8.
5. Mandal S, Mandal MD, Pal NK, Saha K. Synergistic anti-*Staphylococcus aureus* activity of amoxicillin in combination with *Emblica officinalis* and *Nymphae odorata* extracts. Asian Pacific J Trop Med. 2010; 3:711-714.
6. Mandal S, Mandal MD, PalNK. Antibacterial potential of *Azadirachta indica* seed and *Bacopa monniera* leaf extracts against multidrug resistant *Salmonella enteric* serovar typhi isolates. Arch Med Sci. 2007;3:14-18.
7. Rani MS, Dayanand CD, Shetty J, Vegi PK, Kutty AVM. Evaluation of antibacterial activity of *Pongamia pinnata* Linn on pathogens of clinical isolates. American Journal of Phytomedicine and Clinical Therapeutics. 2013;8:645-651.
8. Kirtikar KR, Basu BD. Indian medicinal plants. 2nd edn. Dehra Dun Publisher Ltd. 1995;1:2745.
9. Nadkarni KM: Indian Materia Medica. Bombay, Popular Book Depo. 1954;1: 1001.
10. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. Clinical and Laboratory Standards Institute, Wayne, PA; 2012.
11. Krishna Kumar, Shivasharanappa and Ramesh Londonkar. Isolation, purification and special characterization of flavonoid from fruits of *Ficus glomerata*. World Journal of Pharmaceutical Research. 2014;3:2168-2177.
12. Marathe NP, Rasane MH, Kumar H, Patwardhan AA, Shouche YS, Diwanay SS. *In vitro* antibacterial activity of *Tabernaemontana alternifolia* (Roxb) stem bark aqueous extracts against clinical isolates of methicillin resistant *Staphylococcus aureus.* Ann Clin Microbiol Antimicrob. 2013;12:26.
13. Broekema NM, Van TT, Monson TA, Marshall SA, Warshauer DM. Comparison ofcefoxitin and oxacillin disk diffusion methods for detection of mecA-mediated resistance in *Staphylococcus aureus* in a large-scale study. J Clin Microbiol. 2009;47(1):217-9. DOI: 10.1128/JCM.01506-08. (Epub 2008 Nov 19). PubMed ID:19020073; PMC2620872.
14. Prabha T, Dorababu M, Goel S, Agarwal PK, Singh A, Joshi VK, Goel RK. Effect of methanolic extract of *Pongamia pinnata* Linn seed on gastro-duodenal ulceration and mucosal offensive and defensive factors in rats. Indian J Exp Biol. 2009; 47(8):649-59.
15. Sung WS, Lee DG. The combination effect of Korean red ginseng saponins with kanamycin and cefotaxime against methicillin-resistant *Staphylococcus aureus.* Biol Pharm Bull. 2008;31(8):1614-7.
16. Hossain MA, AL-Raqmi KAS, AL-Mijizy ZH, Weli AM, Al-Riyami Q. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pacific Journal of Tropical Biomedicine. 2013;3(9):705-710.
17. Azevedo MM, Chaves FC, Almeida CA, Bizzo HR, Duarte RS, Campos-Takaki GM, Alviano CS, Alviano DS. Antioxidant and antimicrobial activities of 7-hydroxy-calamenene-rich essential oils from Croton cajucaraBenth. Molecules. 2013;16; 18(1):1128-37.
18. RudiHendra, Syahida Ahmad, and Ehsan Oskoueian. Flavonoid Analyses and antimicrobial activity of various parts of *Phaleria macrocarpa* (Scheff) Boerl Fruit. Int J Mol Sci. 2011;12(6): 3422-3431.
19. Azevedo MM, Chaves FC, Almeida CA, Bizzo HR, Duarte RS, Campos-Takaki GM, Alviano CS, Alviano DS. Antioxidant and antimicrobial activities of 7-hydroxy-calamenene-rich essential oils from *Croton cajucara.* Benth. Molecules. 2013; 18(1):1128-37.
20. Santiago C, Pang EL, Lim KH, Loh HS, Ting KN. Reversal of ampicillin resistance in MRSA via inhibition of penicillin-binding protein 2a by *Acalypha wilkesiana*. Biomed Res Int. 2014;965348.
21. Celaya LS, Alabrudzińska MH, Molina AC, Viturro CI, Moreno S. The inhibition of methicillin-resistant *Staphylococcus aureus* by essential oils isolated from leaves and fruits of *Schinus areira* depending on their chemical compositions. Acta Biochim Pol. 2014;61(1):41-6. (E pub 2014 Mar 18)
22. Buru AS, Pichika MR, Neela V, Mohandas K. *In vitro* antibacterial effects of *Cinnamomum* extracts on common

bacteria found in wound infections with emphasis onmethicillin-resistant *Staphylococcus aureus*. J Ethno-pharmacol. 2014;153(3):587-95.

23. Wang SY, Sun ZL, Liu T, Gibbons S, Zhang WJ, Qing M. Flavonoids from *Sophora moorcroftiana* and their synergistic antibacterial effects on MRSA. Phytother Res. 2014;28(7):1071-6.
24. Broekema NM, Van TT, Monson TA, Marshall SA, Warshauer DM. Comparison ofcefoxitin and oxacillin disk diffusion methods for detection of mecA-mediated resistance in *Staphylococcus aureus* in a large-scale study. J Clin Microbiol. 2009;47(1):217-9.
DOI: 10.1128/JCM.01506-08. Epub 2008 Nov 19. PubMed ID:19020073; PMC2620872.
25. Kagithoju S, Godishala V, Pabba SK, Kurra HB. Swamy S. Anti-bacterial activity of flower extract of *Pongamia pinnata* linn. Anelite medicinal plant. International Journal of Pharmacy and Pharmaceutical Sciences. 2012;4(3):130-32.
26. Yadav PP, Ahamad G, Maurya R. Furano flavonoids from *Pongamia pinnata* fruit. Phytochemistry. 2004;65:439-43.
27. Hao Yin, Si Zhang, Jun Wu. Prenylated flavonoids from *Pongamia pinnata* Z. Naturforsch. 2005;60b:356-58.
28. Grosvenor PW, Supriono A, Gray DO. Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2: Antibacterial and antifungal activity. Journal of Ethnopharmacology. 1995;45:97-111.
29. Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochemical Pharmacology 32, 1141-1148.] [Pathak D, Pathak K, Singla AK. (1991) Flavonoids as medicinal agents -- recent advances. Fitoterapia. 1983;62: 371-389.
30. Pan X, Bligh SW, Smith E. Quinolone alkaloids from Fructus Euodiae shows activity against methicillin-resistant *Staphylococcus aureus*. Phytother Res. 2014;28(2):305-7.
DOI: 10.1002/ptr.4987.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/10465