***Chapter 8***
Print ISBN: 978-93-89246-05-6, eBook ISBN: 978-93-89246-49-0

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

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

DOI:10.9734/bpi/rabr/v3

## ABSTRACT

**Introduction:** Methicillin resistant *Staphylococcus aureus* (MRSA) is defined as the resistant to penicillinase-stable penicillin's, thus the acronym MRSA is still under use even though methicillin is no longer the agent of choice for treatment. The use of vancomycin for MRSA remains as the treatment of choice but concerns with rising resistance to glycol peptides call for the restrictive use of these drugs. The resistance mechanism and the genes that mediate resistance have presumably evolved in organism that produce antibiotics such that the antibiotic produced is not effective against the producing organism.
**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.
**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. Flavonoids occupy the first grade antimicrobials in combating methicillin resistant staphylococcus aureus infections. These infections which are prominent in ICU units and HICU units can be drastically controlled without any side effects.

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

*[1]Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.*
*[2]Department of Biochemistry/Allied Health Sciences, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.*
*[3]Department of Microbiology, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.*
**Corresponding author: E-mail: cd8905@yahoo.co.in;*

## 1. BACKGROUND

Methicillin resistant *Staphylococcus aureus* (MRSA) is defined as the resistant to penicillinase-stable penicillin's, thus the acronym MRSA is still under use even though methicillin is no longer the agent of choice for treatment [1,2]. Resistance to antimicrobial therapies has resulted in substantial increase in morbidity and mortality resulting in treatment failures leading to increased health care costs. Defining the precise health risk and estimation the increased cost is not a simple undertaking, which made the antibiotic resistance a global concern. Widespread use of antibiotics exerts pressure that acts as a driving force in the development of antibiotic resistance. The association between use of antimicrobials and the increased resistance has been well documented for nosocomial infections as well as community acquired infections [3].

The use of vancomycin for MRSA remains as the treatment of choice but concerns with rising resistance to glycol peptides call for the restrictive use of these drugs. The resistance mechanism and the genes that mediate resistance have presumably evolved in organism that produce antibiotics such that the antibiotic produced is not effective against the producing organism. The use of vancomycin is restricted by several issues that include low bactericidal activity and tissue penetration. The other commonly used antibiotics like ceftaroline and daptomycin are becoming resistant. There seems to be a need to identify alternate agents for MRSA treatment based on the site of infection [4,5].

India being the largest countries in population tops up in source of medicinal plants too that accounts for 120 families and 130000 plant species. Since, pre-historic times, variant parts of these plants were used in disease prevention and control. However, the bioactive compounds exhibiting antimicrobial properties are yet to be discovered. Studies focussed on plants contributing anti-MRSA activity were found to be *Punica granatum, Plumbago zeylanica, Emblica officinalis, Camellia sinensis, Lawsonia inermis and Azardirachta indica*. All these studies showed evidence of anti-MRSA activity but there is a lack of information on precise phyto constituents possessing anti MRSA activity. Most of these studies used crude formulations and could not conclude the exact phytochemical constituent pertaining to anti-MRSA activity. Hence, screening of exact phytochemical compound more relevant in treatment of MDR pathogens such as MRSA [6-13].

The present study is aimed to screen the phytochemical compound flavonoid from seeds of *Pongamia pinnata* against MRSA.

## 2. *Pongamia pinnata* Linn

*Pongamia pinnata* is a medium sized tree belongs to family Leguminosae and sub family Papilionaceae. The tree grows in the regions of Asia and Australia. It is known as Karanaja in Hindi, Pungam in Sidha, Indian beech in English and Ponga, Pongam in Tamil. It is native to India, Nepal, Bangladesh, Myanmar and Thailand. Native to humid and sub-tropical environments and thrives in areas of annual rainfall. It grows to elevations of 1200 m but in Himalayan foot hills its height is not found above 600m. It does not grow on dry sands and is highly tolerant to salinity. Highest growth rate is observed on well drained soils with good moisture. It is also known as Pongamia glabra vent, Derris indica bennet, Pongam, Indian beech, Karum tree, Poonga oil tree.

***Pongamia Pinnata* L:**

**Botanical description:**

The plant is medium sized evergreen deciduous tree with 35-40 feet height, fast growing. Leaves are alternate, pinnately compound, 2-4 inches in size. Flowers are Lavender, pink, 2-4 together and 15-18 mm long. Pods are 3-6 cm long and 3 cm wide, thick walled, indehiscent 1-2 seeded. Seeds are ovoid or elliptical, been like, 10-15 cm long, dark brown and oily. Roots are tap root, thick and long, lateral roots are numerous and well developed. Bark is thin and greyish brown and yellow on the inside.

The Phytochemical constituents of plant include alkaloids, glabrins, kaempferol, karangin, pinnatin, pongamol, quercetin and tannin. The seeds are reported to have 19% moisture, 17.4% protein, 6.6% starch, 27.5% fatty oil, 7.3% crude fibre and 2.4% ash. The nutritional value of leaves and twigs are nitrogen 1.16, 0.71; potash 0.49, 0.62; phosphorous 0.14, 0.11; and lime 1.54, 1.58% [14].

**Collection of material:**

*Pongamia pinnata L* seeds were collected from campus of Sri Devaraj Urs Academy of Higher Education and Reseach, Tamaka, kolar. The seeds were de-shelled and good quality used for our investigation. All other chemicals used are of analytical grade.

**Preparation of fine seed powder:**

The seeds material was air dried and powdered.10 grams of fine seed powder was dissolved in 30 mL of methanol in a beaker. The mixture was placed in an orbital shaker for 72 hours, for maceration and then was filtered using whatman filter paper. The filtrate obtained is evaporated and resulted powder is stored in amber coloured air tight bottle and preserved until use.

**Phytochemical investigation:**

Phytochemical investigation of seeds of *Pongamia pinnata* were performed by adopting qualitative tests for the presence of various phytochemicals [15,16].

**Isolation & purification of flavonoids from fine seed extract:**

The fine powdered filtrate of 100 mg/ml was solubilized in methanol and applied to glass column chromatography developed by Silica gel with a mesh size of 60-120 mm and equilibrated and eluted with methanol (2 mL/min). The active fractions were collected in a time interval of 1 min and qualitatively tested for flavonoid content and confirmed fractions were pooled. The pooled fractions were subjected for drying in air, thus obtained powder used for quantitative determination of flavonoids.

**Qualitative detection of flavonoid components:**

Each eluted fractions collected from column chromatography procedure were tested for flavonoid presence using few drops of 20% NaOH to the flavonoid fraction. The appearance of yellow colour which later on turns into colourless upon addition of concentrated hydrochloric acid indicates the presence of flavonoids [17].

**Quantification of flavonoids:**

Quantification of flavonoids was performed by Aluminium chloride method [18]. Stock solution of quercetin (3 mg/ml) was aliquoted into different tubes to obtain the concentration in the range of 50 to 500 µg/ml. Similarly, fine powder obtained from pooled fraction containing total flavonoids was also diluted to obtain the concentration range of 50 to 500 µg/ml. A volume of 0.5 ml of each fraction mixed with 1.5 ml of methanol, 0.1 ml of Aluminium chloride, 0.1 ml of 1M potassium acetate and 1.8 ml of distilled water to get the total volume of 4 ml. Contents were mixed and allowed for 30 minutes incubation in room temperature. The solution was centrifuged, clear supernatant used for measurement of absorbance. The obtained results were graphically represented in the Fig. 1.

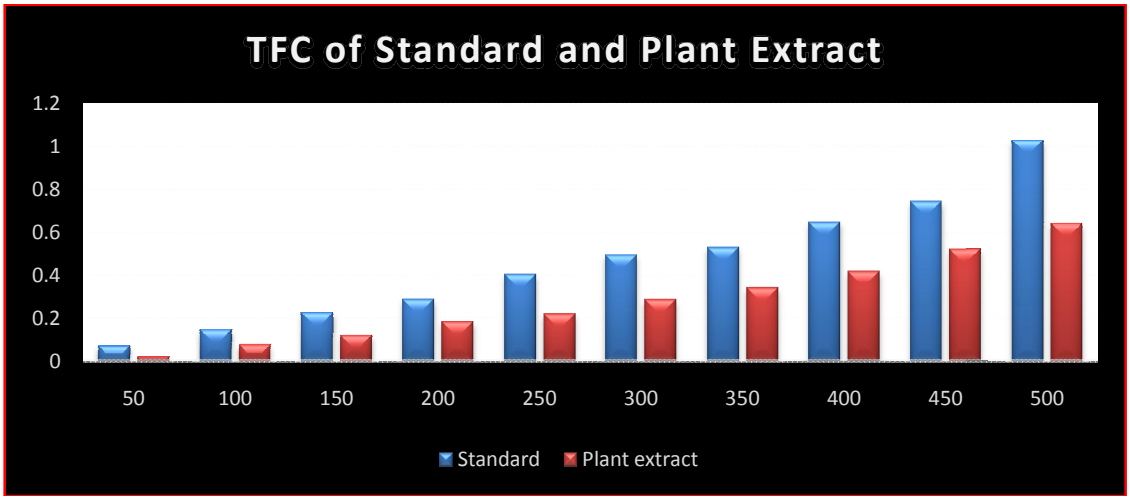


**Fig. 1. Showing the total flavonoid content of standard and sample**

**Preparation of inoculums:**

MRSA isolates were collected from department of microbiology, Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar.

The cultures were grown in nutrient broth at 37°C, maintained in nutrient agar slants and stored at 4°C for determining the antimicrobial activity of flavonoids isolated from seeds of *Pongamia pinnata Linn.*

**Screening of bactericidal activity of flavonoids:**

Antibacterial activity of flavonoid components of *Pongamia pinnata Linn* was performed by Agar well Diffusion method. 100 µl of MRSA inoculum was spread on nutrient agar plate and different concentrations like 25 µg/ml, 50 µg/ml, 100 µg/ml, 200 µg/ml and 400 µg/ml of crude extract of *Pongamia pinnata* as well as isolated flavonoid, quercetin and vancomycin were made by using Dimethyl sulphoxide. 20 µl of each prepared concentrate was added into respective wells inoculated with MRSA. DMSO was used as internal control. All the plates were incubated at 37°C for 24 hours. The zone of inhibition of each concentration was measured and the experiments were performed in triplicates to get good observation [19].

## 3. RESULTS

Since ancient times, plants are considered as richest bio-sources for synthetic drugs as they were used in treating different types of infections. Therefore, modern medicine has evolved from traditional system by application of several chemical process for their phytochemical screening which are used in synthetic drugs [20,21]. The emergence of drug-resistant bacteria is a therapeutic problem. Hence, an attempt was made in the current study to screen for the exact phytochemical compound possessing the antimicrobial activity. In this regard, flavonoids were isolated from seeds of *Pongamia pinnata Linn* and confirmed by qualitative and quantitative tests which showed that, the obtained flavonoid content is almost 25% in total phytochemical constituents.

The results of antibacterial activity revealed that the crude as well as flavonoids isolated contributed significant antimicrobial activity in comparison with the standard antibiotic used vancomycin. About 89% inhibition was noticed in crude and 84% inhibition was in flavonoid extract tested for antimicrobial activity in comparison with vancomycin taken as 100%. Whereas the standard flavonoid quercetin exhibited 92% inhibition compared with vancomycin. The results obtained from the study revealed that quercetin, the widely distributed flavonoid from flavonoid group had conferred maximum antimicrobial activity hence it can be justified that the chief component quercetin might be the inhibiting factor / agent.

Thus, the sensitivity and specificity of crude extract ranges from 25 to 400µg/ml that revealed 75-89% inhibition in comparison with vancomycin. Similarly, flavonoids exhibited 71-925 inhibition in the same concentration with vancomycin. The results were depicted in the below Table 1 and Figs. 2 and 3.

**Table 1. Antibacterial activity of flavonoids against MRSA with different concentrations**

| 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 |

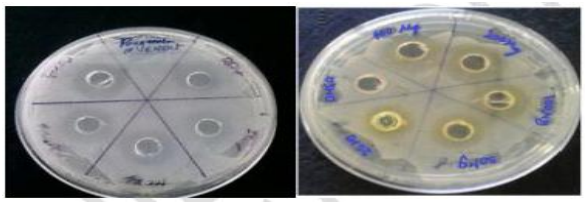

**Fig. 2. Antibacterial activity of crude extract and flavonoids extracted from *Pongamia pinnata***

**Fig. 3. Antibacterial activity of vancomycin and quercetin**

## 4. DISCUSSION

Since, the modern medicine is in threat of losing its efficacy due to persistent resistant mechanism of microorganisms. It is essential to screen for novel drug resources from natural sources as 80% of World population are still dependent on Herbal medicine. Thereupon, an attempt towards identifying molecules having affluent biological properties has drawn assiduity towards the widely distributed secondary metabolites alike flavonoids. Flavonoids being as higher ranked polyphenolics contribute therapeutic benefits due to their molecular structure containing phenol ring. Phenols are the magical compounds with an ability to link with proteins and bacterial membranes to form complexes [22]. Thence, in our study, an attempt has been made to extract and purify the flavonoid from seeds of *Pongamia pinnata* Linn.

Even though vancomycin is considered as the first line antibiotic used for MRSA treatment, it was found that it shows slow onset of bactericidal activity and poor penetration capacity. While many countries like Europe and United states recommend fixed dose with a goal to achieve minimum inhibitory concentration ratio ≥ 400 µg/ml which is in consistent with the current results that flavonoid exhibited good antimicrobial activity with the opted concentration [23]. Flavonoids have been reported widely in all plant species with less toxicity and hence considered in many therapeutic applications. Similarly, the present investigation on flavonoids proved to be an selective inhibitor of MRSA. There could also be an interesting fact that, isolated flavonoids when compared with vancomycin against MRSA showed arbitrarily nearest inhibitory effect which indicates the presence of quercetin in the isolated flavonoids.

Nitiema et al. [24] studied on antibacterial activity of quercetin on commonly encountered pathogens in Intensive care units and High dependency care units viz *S. aureus, E. coli* and *Proteus vulagris* with a concentration of 300-400 µg/ml. The desired concentration had showed significant inhibitory activity on *S. aureus* and *E. coli* which co-ordinates with current study on MRSA, hence can be confirmed that isolated flavonoid has potent amount of quercetin. Secondary metabolites particularily phenolics known to exert bactericidal action [25,26]. Thereupon, the secondary metabolites of plants are proved to contribute benefits in variant kinds to human in combating various diseases.

## 5. CONCLUSION

The conclusion from the present study is that, flavonoids occupy the first grade antimicrobials in combating methicillin resistant *Staphylococcus aureus* infections. These infections which are prominent in ICU units and HICU units can be drastically controlled without any side effects.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. World Health Organization. WHO antimicrobial resistance: Global report on surveillance; 2014.
   Available:http://www.who.int/drugresistance/documents/surveillancereport/en/
   (Accessed 28 Oct 2016)
2. Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States; 2013.
   Available:https://www.cdc.gov/drugresistance/threat-report-2013/
   (Accessed 17 Mar 2016)
3. Ali Hassoun Peter K Linden, Bruce Friedman. Incidence, prevalence and management of MRSA bacterimia crosses patient populations- a review of recent developments in MRSA management and treatment. Crit Care. 2017;21:211.
   DOI: 10.1186/s13054-017-1801-3
4. Jacob JT, DiazGranados CA. High vancomycin minimum inhibitory concentration and clinical outcomes in adults with methicillin-resistant *Staphylococcus aureus* infections: A meta-analysis. Int J Infect Dis. 2013;17:e93–e100. [PMC free article] [PubMed]
5. Kalil AC, Van Schooneveld TC, Fey PD, Rupp ME. Association between vancomycin minimum inhibitory concentration and mortality among patients with *Staphylococcus aureus* bloodstream infections: A systematic review and meta-analysis. JAMA. 2014;312:1552–64. [PubMed]
6. Aqil F, Ahmad I, Owais M. Evaluation of anti-methicillin-resistant *Staphylococcus aureus* (MRSA) activity and synergy of some bioactive plant extracts. Biotechnol J. 2006;1:1093–102. [PubMed] [Google Scholar]
7. Phongpaichit S, Pujenjob N, Rukachaisirikul V, Ongsakul M. Antimicrobial activities of the crude methanol extract of *Acorus calamus* Linn. Songklanakarin J Sci Technol. 2005;27(Suppl 2): 517–23. [Google Scholar]
8. Kar A, Jain S. Antibacterial evaluation of some indigenous medicinal volatile oils. Qualitas Plantarum Materiae Vegetabiles. 1971;20:231–7. [Google Scholar]
9. Kirubanandan S, Swethkamal K, Renganathan S. Activities of triphala towards promoting collagen synthesis at wound site and inhibiting methicillin-resistant *Staphylococcus aureus* and its enzymes. Int J Pharm Pharm Sci. 2013;5:54–62. [Google Scholar]
10. Mehrotra S, Srivastava AK, Nandi SP. Comparative antimicrobial activities of Neem, Amla, Aloe, Assam Tea and Clove extracts against *Vibrio cholerae*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. J Med Plants Res. 2010;4:2473–8. [Google Scholar]
11. Aqil F, Khan MS, Owais M, Ahmad I. Effect of certain bioactive plant extracts on clinical isolates of beta-lactamase producing methicillin resistant *Staphylococcus aureus*. J Basic Microbiol. 2005;45:106–14. [PubMed] [Google Scholar]

12. Hena JV, Sudha S. Studies on community acquired MRSA (Methicillin resistant *Staphylococcus aureus*) and its treatment regimen. Indian J Applied and Pure Bio. 2011;26:63–6. [Google Scholar]
13. Quave CL, Plano LR, Pantuso T, Bennett BC. Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*. J Ethnopharmacol. 2008;118:418–28. [PMC free article] [PubMed] [Google Scholar]
14. Chopade VV, et al. *Pongamia pinnata*: Phytochemical constituents and traditional uses and pharmacological properties: A review. International Journal of Green Pharmacy. 2008;115.184.228.141:72-75.
15. 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.
16. 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.
17. Azevedo MM, Chaves FC, Almeida CA, Bizzo HR, Duarte RS, Campos-Takaki GM, Alviano CS, Alviano DS. Antioxidant and antimicrobial activities of 7-hydroxycalamenene-rich essential oils from *Croton cajucara* Benth. Molecules. 2013;18(1):1128-37.
18. Kalita Palleb, Barman Tapani K, Pal Tapas. Estimation of total flavonoids content (TFC) and antioxidant activities of methanolic whole plant extract of *Biophytum sensitivum* Linn. Journal of Drug Delivary and Therapeutics. 2013;3(4):33-37.
19. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J Biotechnol. 2008;7(12): 1797–1806.
DOI: 10.4314/ajb.v7i12.58804
20. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. African J Biotechnol. 2008;7(12):1797–1806.
DOI: 10.4314/ajb.v7i12.58804
21. Alagesaboopathi C, Sivakumar R. Phytochemical screening studies on the leaves and stem of *Andrographis neesiana* Wight-An endemic medicinal plant from India. World Appl Sci J. 2011;12:307–11.
22. Falleh H, Ksouri R, Chaieb K, Karray-Bouraoui N, Trabelsi N, Boulaaba M, Abdelly C. Phenolic composition of *Cynara cardunculus L*. organs, and their biological activities. C R Biol. 2008;331(5):372-9.
DOI: 10.1016/j.crvi.2008.02.008
23. Rybak M, Lomaestro B, Rotschafer JC, Moellering R. Jr, Craig W, Billeter M, et al. Therapeutic monitoring of vancomycin in adult patients: A consensus review of the American Society of Health-System Pharmacists, the Infectious Diseases Society of America, and the Society of Infectious Diseases Pharmacists. Am J Health Syst Pharm. 2009;66:82–98.
DOI: 10.2146/ajhp080434
24. Nitiema LW, Savadogo A, Simpore J, et al. *In vitro* antimicrobial activity of some phenolic compounds (Coumarin and Quercetin) against gastroenteritis bacterial strains. International Journal of Microbiol Research. 2012;3:183-7.
DOI: 10.5829/idosi.ijmr.2012.3.3.6414
25. Karaaslan M, Ozden M, Vardin H, Turkoglu H. Phenolic fortification of yoghurt using grape and callus extracts, LWT. Food Science Technology. 2011;44:1065-72.
DOI: 10.1016/j.lwt.2010.12.009
26. Karou D, Dicko MH, Simpore J, Traore AS. Antioxidant and antibacterial activities of polyphenols from ethno medicinal plants of Burkina Faso. African Journal of Biotechnology. 2005;4:823-28.

**Biography of author(s)**

**Miss Mary Shobha Rani**
Department of Cell Biology and Molecular Genetics, Sri Devaraj Urs Academy of Higher Education and Research Kolar, Karnataka, India.

She has done masters in Biotechnology, Post graduate Diploma in Genomic Technology and currently pursuing PhD under faculty of Allied Health and Basic Sciences in Sri Devaraj Urs Academy of Higher Education and Research, Kolar. Started carrier as Research Assistant in field of Phytochemistry, Microbiology, Biochemistry, Cell Biology and Cancer Biology with 7 years of experience. Focused thrust areas were isolation of phyto-chemicals and screening of their biological potentials. Contributed extensive research work on flavonoids of *Pongamia pinnata* Linn. Authored 5 research publications indexed in SCOPUS with h-index 1.

**Dr. C. D. Dayanand**
Department of Biochemistry/Allied Health Sciences, Sri Devaraj Urs Academy of Higher Education and Research, Kolar, Karnataka, India.

He pursued M.Sc., Ph.D. in Biochemistry. He is working as a Professor in Department of Biochemistry, Sri Devaraj Urs Academy of Higher Education and Research, Kolar. Member of ACBI, Chairman BOS of Allied Health and basic Sciences and Faculty of Medicine. 25 years of teaching experience for MBBS students, Co-ordinator of Allied Health sciences, M. Phil and Ph. D Guide in Faculty of Medicine and Faculty of Allied Health Sciences, guided 04 M. Phil, 01 Ph.D. student and as. Co-guide for 6MD students in Obstetrics and Gynecology subject. Research thrust areas include Enzymology, proteomics and Pre-eclampsia. Research contribution with 35 publications in peer reviewed Indexed journals, Rated by Research gate with RG score of 12.0 in the scientific community and h - index is 5.0.



**DISCLAIMER**
This chapter is an extended version of the article published by the same authors in the following journal with CC BY license.
British Microbiology Research Journal, 10(1): 1-8, 2015.

**Reviewers' Information**
(1) Anonymous, National University of San Luis, Argentina.
(2) Anonymous, Cumhuriyet University, Turkey.