ISSN: 2278-5213

RESEARCH ARTICLE

# Phytochemical screening and antibacterial activity of *Vigna radiata* L. against bacterial pathogens involved in food spoilage and food borne diseases

Jaya Prakash Priya. A[1], Yamini SudhaLakshmi. G[2], Fouzia Banu[3], Gopalakrishnan. S[4], Dhanalakshmi. P[1], Sagadevan. E[1], Manimaran. A[5] and Arumugam. P[1]

[1]ARMATS Bioproducts Unit, ARMATS Biotek Pvt. Ltd., #14/18, Link Road, Maduvankarai, Guindy, Chennai-600032
[2]Dept. of Biochemistry, Professor Dhanapalan College of Arts and Science, Kelambakkam, Chennai-603103
[3]Dept. of Biochemistry, JBAS College for Women, Chennai, India
[4]Dept. of Applied Sciences, PNG University of Technology, Papua New Guinea
[5]Dept. of Biological sciences, Aarupadai Veedu Institute of Technology, Paiyanoor, Chennai-603104
gingeesaga@gmail.com; +91 9444857864

## Abstract

Chemical preservatives have been used in the food industry for many years. However, with increased health concerns, consumers prefer additive-free products or food preservatives based on natural products. In this context, this study evaluated antibacterial activities of extracts from *Vigna radiata* L. belonging to the Fabaceae family, to explore their potential for use against pathogens causing food borne diseases so that new food preservatives may be developed. Sprouted seeds of *V. radiata* were extracted with three different solvents such as methanol, ethyl acetate and hexane The antibacterial potentiality using solvent extracts were studied by agar well diffusion and broth dilution method against antibiotic resistant food borne or food spoilage pathogenic bacteria. The methanol extracts showed significant concentration dependent antibacterial activity against almost all the test pathogens. Qualitative phytochemical tests and thin layer chromatography of active methanolic extract demonstrated the presence of various phytochemicals in *V. radiata* including glycosides, steroids, phenols, saponins, alkaloids and flavonoids as major active constituents. This study assumes much significance in finding antibacterial phytochemicals from the sprouts of *V. radiata* and the study has to be proceeded further to isolate, purify and characterize the active principle.

**Keywords:** *Vigna radiata,* antibacterial activity, methonolic extracts, phytochemcials, sprouted seeds.

## Introduction

Food poisoning is still being considered as a major concern for both consumers and the industrial producers despite the use of various preservation methods. All over the world, personal from food industries, researchers involved in food safety research and regulatory authorities are continuously concerned with high and growing number of disease outbreaks caused by some pathogenic and spoilage microorganisms in foods. The exposure of foods and damaged cells to the environment attracts microorganisms (e.g. bacteria, molds and virus) and insects, which in turn further accelerate the decomposition of the food. For most food poisoning, spoilage has not reached the stage where the sensory attributes appearance, smell, taste, texture, etc.) of the food are abnormal. Food borne illness also has a major economic impact on society, costing billions of dollars each year in the form of medical bills, lost work time and reduced productivity. Food borne illnesses generally cause temporary disorders of the digestive tract; however, they can also lead to more serious consequences (Blakeslee and Penner, 2006). In recent years, a number of widely reported outbreaks of food borne illness caused by microbial contamination have increased public awareness and concern about the safety of food.

The increasing antibiotic resistance of some pathogens that are associated with food borne illness is another concern (Stermitz *et al*., 2000). Antimicrobial activity of spices and herbs has been known and described for several centuries (Bagamboula *et al*., 2003). There is growing interest in using natural antibacterial compounds, such as extracts of spices and herbs, for food preservation (Smid and Gorris, 1999). Numerous studies have been published on the antimicrobial activities of plant extracts against different types of microbes, including food borne pathogens (Hara-Kudo *et al*., 2004). In the natural environment, seed sprouts survive during germination by enhancing their defensive responses through phenolics biosynthesis including modified vitamins, enzymes, and receptors etc (Randhir *et al*., 2007). Among the enhanced defensive mechanisms during germination, the antimicrobial defenses might be highly involved. However, the antimicrobial defenses were not covered adequately in germinated sprouts in most of medicinal plants. Plant phenolic metabolites are gaining interest due to their potential role in human disease prevention and treatment. The use of phytochemicals as natural antimicrobial agent commonly called ‘biocides’ are gaining popularity (Smid and Gorris, 1999).

Mung bean sprout (*Vigna radiata* L.) or previously known as *Phaseolus radiatus*, which is popular in Asian cuisine, is an excellent source of vitamins, minerals and protein with its essential amino acid profile comparable to that of soybean and kidney bean (Mubarak, 2005). Mung bean contains significant quantities of phenolic and polyphenolic compounds such as phenolic acids and flavonoids (Dabrowski and Sosulski, 1984a and b). However, the sprout of mung bean has received little attention in view of phytochemcial analysis and bioactivity screening. Hence, this work has been attempted to study the antimicrobial activities of mung bean sprout against food borne pathogens and food spoilage bacteria. Further, the crude extracts of mung bean sprout were also profiled for phytochemicals with the ultimate focus of establishing new methods of producing safe foods that have a natural or green image.

## Materials and methods

*Plant material:* The fresh Mung bean sprouted seeds, (*Vigna radiata* L.) were purchased from local markets in Chennai, Tamil Nadu. The sprouted seeds were carefully washed with tap water, rinsed with distilled water and air-dried for 1 h. Then the seeds were shade dried in room temperature for one week. Then they were ground into powder and subjected to extraction with different solvents.

*Preparation of extracts from V. radiata sprouts:* The ground material was extracted with different solvents such as, methanol, ethyl acetate and hexane (Eloff, 1998a). The extracted sprouted seed's powder was done with solvents in the ratio of 1:10 under shaking condition. The extracts were collected in different conical flasks and the same was repeated thrice to attain maximum extraction. Then the solvents were condensed to concentrate the extracts obtained. The concentrated extracts were weighed and re-dissolved in respective solvents to yield 10 mg/mL solutions for further analysis.

*Antibacterial activity*

*Microorganisms:* The following bacterial strains, which cause food poisoning and food borne diseases, were used as test pathogens *viz., Escherchia coli, Salmonella typhi, Klebsiella pneumoniae, Proteus vulgaris* and *Streptococcus faecalis.* Cultures were obtained from the Culture Collection of Armats Biotek Training and Research Institute, Guindy Chennai, India and maintained in Nutrient Agar at refridgerated conditions until usage. Subcultures were made in the same media.

*Well diffusion assay:* Nutrient agar was prepared and poured in the petri dish 24 h growing cultures were swabbed on it. Wells (10 mm diameter) were made by using cork borer and the different concentrations of the crude extract were loaded in the wells. The plates were then incubated at 37ºC for 24 h. The inhibition diameter was then measured (Eloff, 1998b).

*Broth dilution assay:* Dilution assays are standard method used to compare the inhibition efficiency of the antimicrobial agents. 5 mL of the nutrient broth, 0.1 mL of the 24 h growing culture (*E. coli, S. typhi, K. pneumoniae, P. vulgaris* and *S. faecalis*) and the different concentrations (100 to 1000 µg) of the drug dissolved in dimethyl sulphoxide was added in the test tubes. The tubes were incubated at 37ºC for 24 h. The optical density was measured spectrophotometrically at 600 nm. The percentage of viable cells was calculated using the following formula (Cos *et al.,* 2006).

$$\% \text{ of viable cells} = \frac{\text{Control OD} - \text{Test OD}}{\text{Control OD}} \times 100$$

*Thin layer chromatography:* The methanol extract of sprouted *V. radiata* was loaded on pre-coated silica plates which were then developed using the solvents methanol, chloroform in the ratio of 1:9. The spots were identified both in the UV light, fluorescent light and in the iodine chamber. Then, $R_f$ value was calculated as the ratio of distance traveled by the solute to the distance traveled by the solvent.

*Phytochemical screening of V. radiata crude extract:* Qualitative phytochemical tests for the identification of alkaloids, phenols, flavonoids, terpenoids, steroids and saponins were carried out for all the extracts by the method described by (Harborne, 1998; Sazada *et al.,* 2009). Freshly prepared extracts of *V. radiata* was qualitatively analysed for the presence of chemical constituents.

## Results and discussion

The phytochemical analysis and antimicrobial evalualtion of *V. radiata* is an attempt to unravel the scientific benefits (if any) derivable against food pathogens. The microbes used in this study were selected on the basis of their implication as agents of food spoilage and food–borne diseases.

*In vitro* preliminary screening of the antibacterial activity against food pathogens from solvent extracts of sprouted *V. radiata* was studied by agar well diffusion method. Plants are known to produce secondary metabolites that complement their structural barrier in their efforts at warding of microbial attack (De Lucca *et al*., 2005). Farooq *et al*. (2007) reported that plants occur in varying habitats, a great magnitude of variation in the concentration and composition of phytochemical ingredients in the different parts of such plant is expected. Moreover, Waller and Nowacki (1978) reported that phytochemicals are produced in response to perceived threats by the plants, therefore variation exist in the production of these phytochemicals depending on the type and amount of threat encountered by the plant.

A number of the secondary metabolites are associated with different potentials in various plants (Smith *et al.*, 2001; Ojekale *et al.*, 2006) including antibacterial properties (Duraipandiyan *et al.*, 2006), even though most of these have been conducted at the *in vitro* level (Cowan, 1999). The antimicrobial screening of *V. raidate* sporut extracts revelaed various levels of bioactivity on food spoilage organisms such as *E. coli, S. typhi, K. pneumoniae, P. vulgaris* and *S. faecalis* and this is much encouraging as these organisms range from pathogenic and toxigenic organisms liable to cause food-borne illnesses to spoilage-causing organisms liable to spoil food products. The control of these organisms by the extracts in foods would reveal the potentials of these extracts as preservatives. The findings add impetus to the clarion call by consumers and authorities in food industries for the replacement of chemically synthesized sanitizers/preservatives with "naturally derived" ones (Jancxsen *et al*., 2002; Lanciotti *et al*., 2003).

Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effects. As a result, some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance. Successive isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. The traditional healers use primarily water as the solvent, but we found in this study that plant extracts prepared with methanol and ethanol as solvents provided more consistent antimicrobial activity as also reported earlier (Allero and Afolayan, 2006; Parekh and Chanda, 2007). Therefore, we used methanol as a solvent to extract almost all of the proposed antimicrobial agents in order to prepare the basis for monitoring different antimicrobial agents as a prelude for the future separation of single antimicrobial compound(s). In this study, among the three extracts studied, methanolic extracts at all the concentrations have inhibited the growth of pathogens effectively when compared with other two solvent extracts. Increased zone of inhibitions were observed when the concentrations of solvent extracts increased (Figs. 1-3). In this study, a maximum of 21 mm zone of inhibition was observed agains *S. faecalil* by 100 µg of crude methanolic extracts. Similar to our report, the *V. mungo* methanol crude extract revealed highly significant antibacterial effect against all the tested gram negative bacteria (Hafidh *et al*., 2011). Preethi *et al*. (2010) also reported that the methanol extraction of *H. antidysenterica* showed high activity on the pathogens above 16 mm inhibition zone. Deepa *et al*. (2012) evaluated the antimicrobial activity of methanol and aqueous extract of *S. emarginatus* by well diffusion against five bacterial and four fungal species in methanol extract showed maximum 29 mm of inhibition zone establish at 120 µg of extract against *Pseudomonas aeruginosa*.

Fig. 1. Antibacterial activity of crude methanolic extracts of *V. radiata* sprouts against food borne pathogenic bacteria.

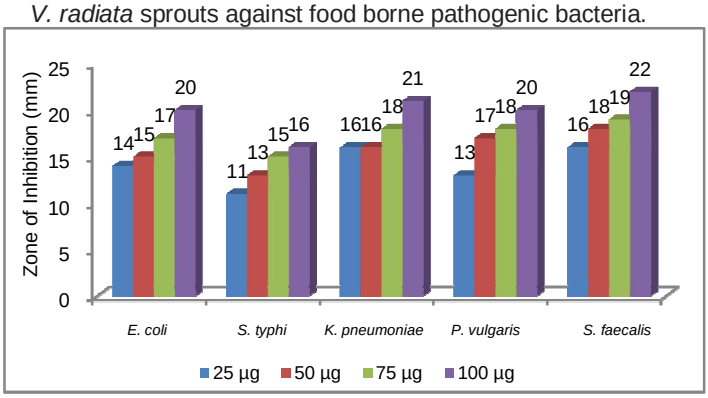



Fig. 2. Antibacterial activity of crude ethyl acetate extracts of *V. radiata* sprouts against food borne pathogenic bacteria.

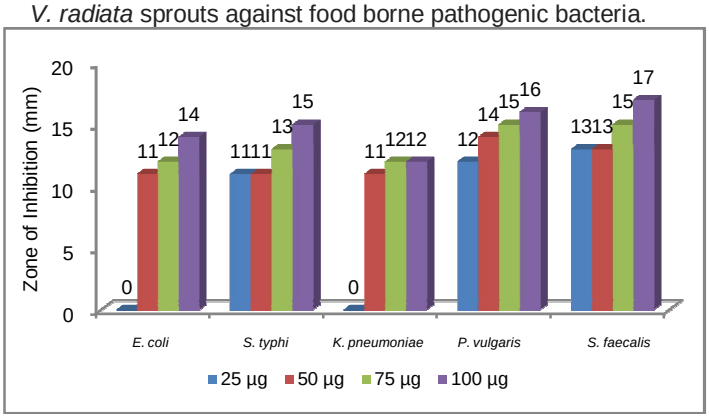
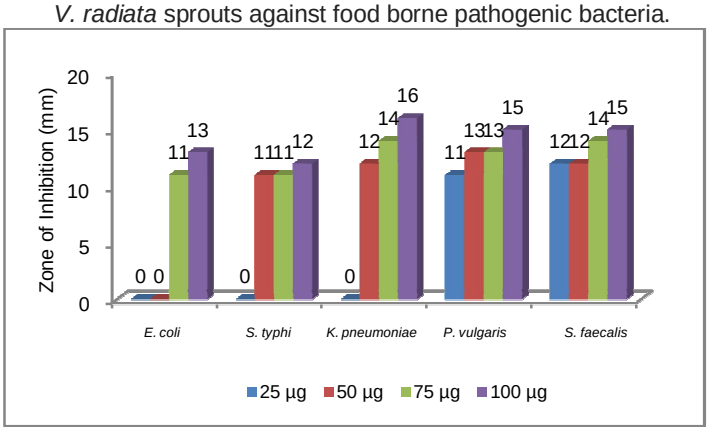



Fig. 3. Antibacterial activity of crude hexane extracts of *V. radiata* sprouts against food borne pathogenic bacteria.




Since methanolic extracts shows maximum inhibition against the pathogens and choosen to determine inhibitory concentration 50 ($IC_{50}$) by broth dilution method. The $IC_{50}$ concentration of methanolic extract of sprouted *Vigna radiata* L. was ranged from 400–600 µg/mL, which was dose dependent manner with different test pathogens (Fig. 4). Nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds. Therefore, they are most often obtained through initial ethanol or methanol extraction (Ahmad *et al*., 1998). MBS is attributed to its ability to create acidic environment.

Fig. 4. Determination of $IC_{50}$ values for crude methanolic extracts of *V. radiata* by broth dilution assay against food borne pathogens (% inhibition).

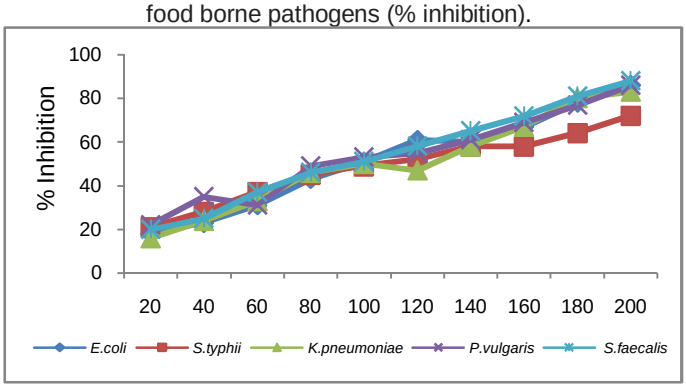



Table 1. Preliminary phytochemical screening of the methanol extract of sprouted *V. radiata*.

| Constituents | Methanol extract |
|---|---|
| Glycosides | ++ |
| Steroids | +++ |
| Phenols | ++ |
| Saponins | + |
| Alkaloids | +++ |
| Flavonoids | +++ |

Seed sprouts have long been used in the diet as health food and recent research shows that in addition to being a good source of basic nutrients, they also have important phytochemicals with disease preventive and health promoting properties (Kurtzweil, 1999). Moreover, germinating seeds or sprouts are believed to have stronger defenses and metabolic pathways than the parent seeds (Mwikya *et al*., 2001; Fernandez-Orozco *et al*., 2008).

This study assumed that one of the enhanced defense mechanisms and modified phytochemical activities in sprouts might be the synthesis of competent antimicrobial phytochemicals that might share their antimicrobial effect with human and animal pathogens. Previous studies have isolated a combination of antimicrobial protein from mung bean which appeared to be active against a range of bacteria and fungi (Wang *et al*., 2004a, b, 2005, 2006; Ye and Ng, 2005; Lin *et al*., 2007). However, few or no studies have focused on the phytochemical profile of the mung bean or mung bean sprout, such as the current methanol extract of MBS, as antimicrobial agents. The chromatogram developed with methanol and chloroform in the ratio of 1:9 revealed the presence of seven major compounds with $R_f$ values of 0.33, 0.42, 0.60, 0.69, 0.75, 0.84 and 0.87 as visualized under iodine vapour and UV illumination. The phytochemical analysis conducted on extracts of sprouted *V. radiata* seeds revealed the presence of glycosides, steroids, phenols, saponins, alkaloids and flavonoids (Table 1).

Further investigations on the active antimicrobial components in the MBS methanol crude extract are necessarily required to provide the pharmaceutical companies with cheap, effective, and most likely novel single antimicrobial agent(s) active against gram positive and negative bacteria. Moreover, germinating seeds, or sprouts, are believed to have stronger defenses and metabolic pathways than the parent seeds (Mwikya *et al*., 2001; Fernandez-Orozco *et al*., 2008). This study assumed that one of the enhanced defense mechanisms and modified phytochemical activities in sprouts might be the synthesis of competent antimicrobial phytochemicals that might share their antimicrobial effect with human and animal pathogens.

## Conclusion

A variety of microorganisms lead food into spoilage that is encountered as one of the most important matter concerning the food industry. So far, many pathogenic microorganisms have been reported as the causal agents of food-borne diseases and/or food spoilage (Deak and Beuthat, 1996). Raw and/or processed foods are open to contamination during the production, sale and distribution of food (Deak and Beuthat, 1996). Due to the economical impacts and the consumer's concerns over safety, the reliability of synthetic chemicals used for treating foods has been in question. Thus, a lot of attention has been paid to naturally derived components or natural products (Alzoreky and Nakahara, 2003). In conclusion, sprouted seeds of *V. radiata* was found to have important antimicrobial activity against pathogens which causing food spoilage and food borne diseases. In this regard the use of this plant and their phytochemicals as natural preservatives in food products and it may be an alternative to the use of chemical additives in the food industry. The results of the study indicate that there is immense potential to develop these antimicrobial principles as natural herbal preservatives. It is suggested that more research are to be conducted to elucidate and characterize the active components and possible mechanism involved in the use of this plant in the food preservation.

## Acknowledgements

The authors are grateful to the Management of Armats Biotek, Chennai for providing the laboratory facilities to carry out the work successfully.

## References

1. Ahmad, I., Mehmood, Z. and Mohammad, F., 1998. Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethnopharmacol*. 62: 183-193.
2. Allero, A.A. and Afolayan, A.J. 2006. Antimicrobial activity of *Solanum tomentosum*. *Afri. J. Biotechnol*. 5. 369-372.
3. Alzoreky, N.S. and Nakahara. K. 2003. Antimicrobial activity of extracts from some edible plants commonly consumed in Asia. *Int. J. Food Mictrobiol*. 62(2): 183-193.

4. Bagamboula, C.F., Uyttendaele, M., and Debevere, J. 2003. Antimicrobial effect of spices and herbs on *Shigella sonnei* and *S. flexneri. J. Food Prot*. 66: 668-673.
5. Blakeslee, K. and Penner, K.P. 2006. Microorganisms and food-borne illness, Kansas State University Publication.
6. Cos, P., Vlietinck, A.J., Berghe, D.V. and Maes, L. 2006. Anti-infective potential of natural products: How to develop a stronger in vitro 'proof-of-concept'. *J. Ethnopharmacol*. 106: 290-302.
7. Dabrowski, K.J. and Sosulski, F.W. 1984a. Composition of free and hydrolyzable phenolic acids in defatted flours of 10 oilseeds. *J. Agric. Food Chem*. 32: 128-130.
8. Dabrowski, K.J. and Sosulski, F.W. 1984b. Composition of free and hydrolyzable phenolic acids in the flours and hulls of ten legume species. *J. Agric. Food Chem*. 32: 131-133.
9. De Lucca, A.J, Cleveland, T.E. and Wedge, D.E. 2005. Derived antifungal proteins and peptides. *Canadian J. Microbiol*. 51(12): 1001–1014.
10. Deak, T. and Beuthat, L.R. 1996. Handbook of food spoilage. New York, USA: CRS Press.
11. Duraipandiyan, V., Ayyanar, M. and Ignacimuthu, S. 2006. Antimicrobialactivity of some ethnomedicinal plants used by Paliyar tribe from Tamil Nadu, India. *BMC Complimentary Alternative Med*. 6: 35.
12. Eloff, J.N. 1998a. Which extractant should be used for the screening and isolation of antimicrobial components from plants? *J. Ethnopharmacol*. 60: 1-8.
13. Eloff, J.N. 1998b. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica.* 64: 711-713.
14. Farooq, A., Sajid, L., Muhammad, A. and Anwarul Hassan, G. 2007. *Moringa oleifera*: A food plant with multiple medicinal uses. *Phytother. Res.* 21: 17–25.
15. Fernandez-Orozco, R., Frias, J., Zielinski, H., Piskula, M.K., Kozlowska, H. and Vidal-Valverde, C. 2008. Kinetic study of the antioxidant compounds and antioxidant capacity during germination of *Vigna radiata* cv. Emmerald, glycine max cv. Jutro and glycine max cv. *Merit. Food Chem*. 111: 622-630.
16. Hara-Kudo, Y., Kobayashi, A., Sugita-Konishi, Y. and Kondo, K. 2004. Antibacterial activity of plants used in cooking for aroma and taste. *J. Food Prot*. 67: 2820-2824.
17. Harborne, J.B. 1998. Phytochemical methods: A guide to modern techniques of plant analysis. 3rd ed. Chapman and Hall, London ISBN: 0-412-57270-2, p.302.
18. Jancxsens, L., Devlieghere, F. and Debevere, J. 2002. Temperature dependence of shelf-life as affected by microbial proliferation and sensory quality of equilibrium modified atmosphere packaged fresh produce. *Postharvest Biol. Technol*. 26: 59–73.
19. Lanciotti, R., Patrignani, F., Bagnolini, F., Guerzoni, M.E. and Gardini, F. 2003. Evaluation of diacetyl antimicrobial activity against *E. coli, L. monocytogenes* and *S. aureus*. *Food Microbiol*. 20: 557–543.
20. Mubarak, A.E. 2005. Nutritional composition and anti-nutritional factors of mung bean seeds (*phaseolus aureus*) as affected by some home. *Trad. Proc. Food Chem*. 89: 489-495.
21. Mwikya, S.M., Camp, J.V., Rodriguez, R. and Huyghebaert, A. 2001. Effects of sprouting on nutrient and anti-nutrient composition of kidney beans (*Phaseolus vulgaris* var. Rose coco). *Eur. Food Res. Technol*. 212: 188-191.
22. Ojekale, A.B., Adeleke, T., Ogundairo, E. and Ordiah. P. 2006. Antimicrobial activities of crude and partial fractions of aqueous and ethanolic leaf and seed extracts of *Carica papaya. Biol. Environ. Sci. J. Tropics.* 3(1): 134–138.
23. Parekh, J. and Chanda, S. 2007. *In vitro* screening of antibacterial activity of aqueous and alcoholic extracts of various Indian plant species against selected pathogens from Enterobacteriaceae. *Afr. J. Biol. Res*. 1(6): 92-99.
24. Preethi, R., Devanathan, V. and Loganathan, M. 2010. Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. *Adv. Biol. Res*. 4(2): 122-125.
25. Randhir, R. and Shetty, K. 2007. Mung bean processed by solid-state bioconversion improves phenolic content and functionality relevant for diabetes and ulcer management. *Innov. Food Sci. Emerg. Technol.* 8: 197-204.
26. Sazada, S., Verma, A., Rather, A.A., Jabeen, F. and Meghvansi, M.K. 2009. Preliminary phytochemicals analysis of some important medicinal and aromatic plants. *Adv. Biol. Res.* 3:188-195.
27. Smid, E.J. and Gorris, L.G.M. 1999. Natural antimicrobials for food preservation. In: Rahman, M.S. (Ed.), Handbook of Food Preservation. Marcel Dekker, New York, pp. 285-308.
28. Smith, M.L., Kobayashi. H., Gawienowski, M. and Briskin., D.P. 2001. An *in vitro* approach to investigate medicinal chemicals synthesis by three herbal plants. *Plant Cell, Tissue Organ Culture*. pp.1–7.
29. Stermitz, F.R., Tawara-Matsuda, J., Lorenz, P., Mueller, P., Zenewicz, L. and Lewis, K. 2000. 5′-methoxyhydnocarpin-D and pheophorbide A: berberis species components that potentiate berberine growth inhibition of resistant *Staphylococcus aureus*. *J. Natural Prod*. 63: 1146-1149.
30. Waller, G.R. and Nowacki, E.K. 1978. Role of alkaloids. Alkaloid biology and metabolism in plants. Plenus press, New York. p.249.