J. Indian Chem. Soc.,
Vol. 92, June 2015, pp. 886-890

Phytochemical and antimicrobial studies of green leafy vegetables and its enhanced bioactive properties upon fortification with probiotic *Lactobacilli acidophilus*

Rachit Pandey[a], Raunak Hisaria[a], Rajdeep Roy[a], E. Nalini[a], V. Vijayakumar[b], K. M. Gothandam*[a] and S. Karthikeyan*[a]

[a]HTS Labs, School of Bio Sciences and Technology, VIT University, Vellore-632 014, Tamilnadu, India

E-mail : skarthikeyan@vit.ac.in

[b]Centre for Organic and Medicinal Chemistry, School of Advanced Sciences, VIT University, Vellore-632 014, Tamilnadu, India

Abstract : In this study we have analyzed the chemical composition of 5 locally available underutilized green leafy vegetables (GLV) obtained from Vellore region, namely : Methi, Mint, Lunia, Amaranthus and Tamarind (common names). Aqueous extracts of GLV's were analysed for various chemical components such as minerals, carbohydrates, proteins, phenols, flavonoids. Antioxidant activity of these components have been evaluated by DPPH method and found to be in the order of Mint > Amaranthus > Tamarind > Lunia > Methi. Mineral contents were measured for all the GLV. All the extracts were tested for their antimicrobial efficacy against Gram-positive and Gram-negative microorganisms. Fortification of extracts with probiotic *Lactobacillus acidophilus* (LAB) was carried out and tested for their antimicrobial property. Upon fortification, it was observed higher antimicrobial property when compared against GLV extracts. This is a novel study carried out for the first time using bio-fortification of phytochemical extracts enhanced LAB strains.

Keywords : Green leafy vegetables, antioxidant, antimicrobial activity, probiotic formulation.

Introduction

The term 'green leafy vegetables' is used to refer leaves of plants that are edible and widely consumed as vegetables. These leafy vegetables are typically low in calories and fat, high in protein content, dietary fibre, minerals, phytochemical contents such as flavonoids, phenolics, terpenoids and vitamins. Dasgupta[1] reported the importance of antioxidants for the human health in a life. Minerals serve three main roles firstly; they provide structure in forming bones and teeth. Secondly, they help to maintain normal heart rhythm, muscle contractility, neural conductivity, and acid-base balance. Lastly, they help to regulate cellular metabolism by becoming a part of enzymes and hormones that modulate cellular activity.

Vegetables are a rich source of vitamins and other components that contribute to antioxidant activity in the diet, while no single vegetable has been found to posses all sources of nutrients required for our day today demands. A diversified diet is needed to meet daily micronutrient requirements[2]. Available data for the micronutrient content and antioxidant activity of some GLV's are for raw samples. Various literature surveys has show caused various analytical procedures which have been used for the determinations of proximate compositions and micronutrients, as well as other important components of GLV's. Health benefits of GLV have been widely described in recent publications especially in prevention of disease associated with oxidative stress, such as cancer and cardiac diseases[3]. Extensive studies have been performed to determine antimicrobial property of various GLV metabolites such as polyphenols, terpeniod, flavanoids etc. on various intestinal pathogenic microbes[4]. However very few studies have been reported for the antimicrobial properties of GLV extracts on other pathogenic microbes. *S. grandiflora* extracts have been reported to have wide antimicrobial property against some of the Gram-positive and Gram-negative bacteria. There are many factors that may influence the survival of probiotics in food such as the physiological state of the added

probiotic in food, physiochemical conditions of food processing and the physical conditions of product storage. In addition, the chemical composition of the product is an important factor to be taken into account. Lastly, interactions with other product components are required to determine how these interactions affect the survival of probiotics. Various studies have discussed development and formulation of probiotics, in this study we aim at developing enhanced probiotic property with the help of plant conjugates.

Results and discussion

Aqueous extracts of all the five green leafy vegetables namely Fenugreek or Methi, Mint, Lunia, Amaranthus and Tamarind (Table 1) was extracted using Soxhlet apparatus. Water based extraction was carried out at little lower temperature of 60 ºC considering the fact that the same conditions have been used in day to day cooking procedure[5]. When we cook at higher temperatures, most of the protein content and other nutrients will get denatured or lose their physiochemical properties, which results in the malnourished content of the food. All the extracts were screened and analysed for the presence of various phytochemical properties they posses, using various standard operating procedure described in experimental procedures.

Mineral analysis of GLV extracts :

Analysis of minerals for ferrous, manganese, magnesium, sodium and potassium was carried out using AAS under standard condition. It is observed that Tamarind, Fenugreek, Methi and Amaranthus leaf extracts had very high ferrous ion content. These contents are quite high when compared to the minimum requirements advised for daily intake[6]. Similarly, we observed very high contents of manganese and magnesium in all the GLV extracts except in Mint and Lunia. It was also observed that Na^+, K^+ and Ca^+ in all the GLV extracts are quite high as per the required dietary demands of an average person[7]. From the above results we strongly recommended that the locally available GLV's can be considered for daily consumption as they have been neglected and underutilized food sources. This will also lead to overcome food insecurity problem by increased consumption.

Total protein, phenol, flavonoids and carbohydrate analysis of GLV extracts :

Crude extracts of GLV's were also analysed for proteins, total phenolic, flavonoids and carbohydrates contents. 1 to 7 g/100 g of protein have been reported for most of the edible green leafy vegetables. Crude extracts when quantified for the carbohydrate quantification, it was observed that the total quantity of carbohydrate ranged from 2.26 to 7.65 g/100 g of leaves. Total content of phenolics and flavonoids for all the five plants were in the following order. For flavonoids : Mint > Amaranthus > Lunia > Fenugreek > Tamarind. For phenolics : Fenugreek > Tamarind > Amaranthus > Lunia > Mint (Table 2).

Antioxidant properties of GLV extracts :

Antioxidant activity at different concentration activity is as follows : Mint > Amaranthus > Tamarind > Lunia > Methi. So now Mint and Amaranthus can be recommended for the antioxidant rich food for the strengthen-

Table 1. Selected green leafy vegetables from Vellore region

Local name	English name	Scientific name	Yield of extract (g)
Vendhaya Keerai	Fenugreek or Methi	*Trigonella foenum-graecum*	7.59
Pudina	Mint	*Mentha spicata*	5.54
Paruppukeerai	Lunia	*Portulaca oleracea*	5.14
Sirukeerai	Amaranthus	*Amaranthus polygonoides*	7.39
Imli	Tamarind	*Tamarind indica*	7.09

Table 2. Total carbohydrate, flavonoids, phenolics, protein content in plant extracts (g/100 g)

Plant	Carbohydrate	Flavonoids	Phenolic	Protein
Fenugreek	7.65	10.00	15.76	7.65
Mint	2.26	47.50	3.59	19.35
Lunia	3.27	18.00	10.47	10.60
Amaranthus	4.37	34.50	13.58	14.85
Tamarind	5.21	5.00	14.28	10.53

ing of immunity and resistance in the body. It will be a potential aid for scavenging free radicals in the body and thus helping to control ageing and other disorders[8].

FTIR analysis of GLV extracts :

The FT-IR spectra of crude extracts of GLV's are shown in Figs. 1, 2, 3, 4 and 5. The FT-IR spectrum of confirms the presence of functional groups for phenolics and flavonoids, which are widely reported for their antioxidant potential. Flavonoids and phenolic acids have antibacterial, antifungal, antiviral, hepatoprotective, immuno modulating and anti-inflammatory properties[9].

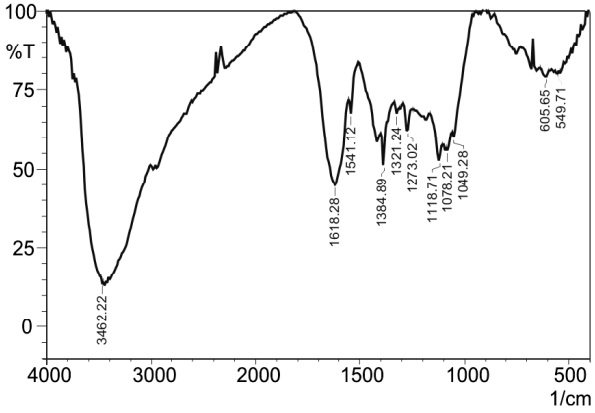

Fig. 1. FTIR of Fenugreek.

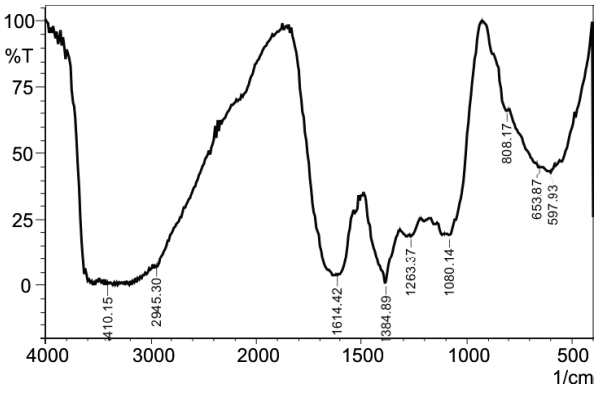

Fig. 2. FTIR of Mint.

Antimicrobial studies of GLV extracts and fortified GLV extracts with probiotic L. acidophilus :

Antimicrobial activity of extracts and extracts of biofortified LAB strains were carried out using disc diffusion method. The results (Table 3) revealed that both the extracts and LAB conjugated with various GLV extracts had potent antimicrobial property against the most common pathogen chosen. The results are considered important since all the organisms tested are important pathogens in humans and animals and were resistance to other drugs as reported in many literatures. While studying growth of *Lactobacilli* in presence of plant extracts along with MRS growth media, samples of Fenugreek and Tamarind showed more protein content when compared with others. Thus indicating that the plant samples had en-

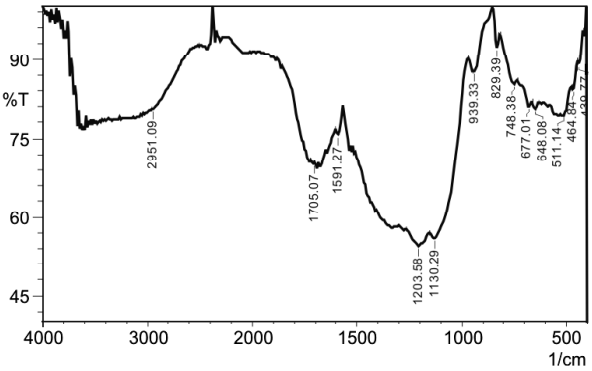

Fig. 3. FTIR of Lunia.

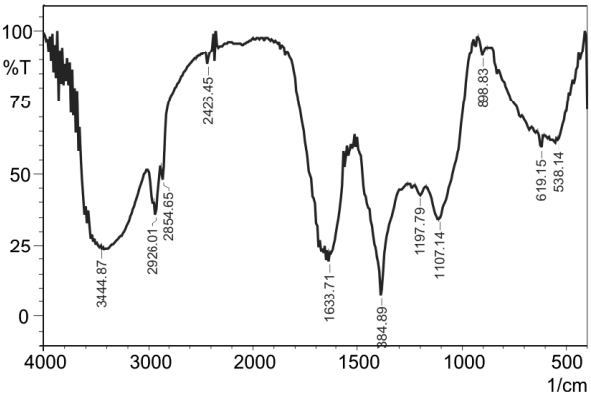

Fig. 4. FTIR of Amaranthus.

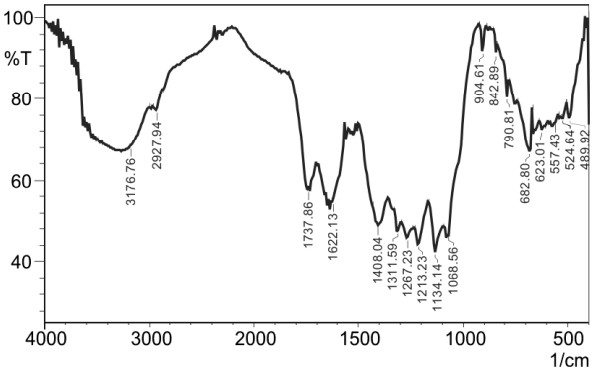

Fig. 5. FTIR graph of Tamarind.

Table 3. Antimicrobial activity of plant extracts against various microorganisms and its zone of inhibition (mm)

Extracts	*E. coli*	*Bacillus*	*Staphylococcus*	*Streptococcus*
Fenugreek	1.2	1.25	1.18	1.5
Fenugreek conjugate	1.3	1.35	1.27	**1.7**
Mint	1.5	1.6	1.44	1.9
Mint conjugate	**1.7**	**1.7**	**1.65**	**2.1**
Lunia	1.3	1.35	1.25	1.3
Lunia conjugate	**1.45**	**1.5**	1.4	1.3
Amaranthus	1.4	1.4	1.3	1.4
Amaranthus conjugate	**1.6**	**1.58**	**1.42**	1.5
Tamarind	1.25	1.3	1.2	1.4
Tamarind conjugate	1.4	1.4	1.3	1.4
LAB	1.2	1.2	1.1	1.1

hanced the growth *Lactobacilli*, hence the bioavailability of *Lactobacilli* was increased. Also there was no suppression of *Lactobacilli* due to plant samples thus a positive note for the probiotic formulation (Table 4).

Table 4. Protein content of fermented broths of LAB with extracts of Fenugreek and Tamarind

Sample	Protein concentration (g/100 g)
Fenugreek extracts	7.65
Tamarind extracts	10.53
Lactobacillus acidophilus	10.60
LAB + Fenugreek	19.35
LAB + Tamarind	14.85

Experimental

Sample collection :

Plant samples were obtained from the Vellore market and were first washed thoroughly to remove presence of any dirt and sand were then crushed and used for Soxhlet extraction. Selected plant samples for our project are given in Table 1. All the extracts were quantified for the presence of total carbohydrate, phenolic, flavonoid and protein contents.

Antimicrobial activity of green leafy vegetable aqueous extracts :

The *in situ* effects of plant different GLV extracts[10] were evaluated against *E. coli, B. subtilis, S. aureus, S. pyogenes* by disc diffusion method and were observed for its antimicrobial activity.

Fourier transform infrared spectroscopy :

FT-IR is the most powerful tool for identifying types of chemical bonds. The FT-IR spectra of GLV extracts were recorded on a Perkin-Elmer 2000 model FT-IR spectrometer (Thermo Fisher Scientific Inc., MA and USA). The dried sample was ground with potassium bromide powder and pressed into pellet for spectrometric measurement in the frequency range of 4000–400 cm^{-1} [11].

Bio-fortification and growth promoting property of the phytochemical extracts of GLV's by co-culturing method :

The prebiotic property of GLV extracts were considered for studying its impact on the probiotic bacterium *L. acidophilus*. The following experimental protocol was set up to fortify *L. acidophilus* strain with phytochemical compounds of GLV's. 0.2 g of all the extracts were sterilized along with MRS medium and were sub cultured with *L. acidophilus*. *L. acidophilus* grown in the above method was checked for their antimicrobial property as per the above experiment.

Conclusion

The following are the highlights of the present study carried out with unutilized green leafy vegetables and their extracts. Consumption of GLV's would help in acquiring hither micro nutrient levels even higher than those found in most exotic leafy vegetables. The GLV's are possessing higher protein content and can overcome protein malnutrition problems. The GLV's are found to possess very important mineral ions such as Fe, Mg, Mn and Ca which are very important in the cellular physical development of all the cells of the body. GLV's were also

found to possess antioxidant properties with varying phenolic and flavonoids contents, resulting use them as cheap natural sources for reducing cellular oxidative damage and consequently reducing degenerative conditions such as cardiovascular and cancers[12]. An affordable probiotic was developed with the help of the conjugation of phytochemical compounds and *Lactobacilli*. This probiotic was tested for its nutritional content and its antimicrobial activity which showed positive results. The economically downtrodden people can now easily afford food products at a cheaper rate and of better quality. This probiotic could be recommended as a diet intake for better health care and strengthening immunity thereby reducing the number of people who get infected with diseases. This will help the poor sections of the society to easily manage their health and they will be no longer deprived of a rich nutritional diet. The analysis of these plants can be also seen in a way of diet recommendation for health benefits. Health-related problems can now be sought out at ease. The cost difference between the normal market probiotic and our probiotic is very huge; ours being a very minimal amount. Homemade curd with easily available plant products could easily be obtained and managed. Health awareness is needed to let people know about the prospective impact of this probiotic. Further research will be carried out to evaluate the potential properties of plant extracts co-culturing with LAB strains and providing affordable probiotic food formulations.

Acknowledgement

The authors gratefully acknowledge VIT University for providing the necessary facility to carry out this work.

References

1. N. Dasgupta and B. De, *Food Chem.*, 2004, **88**, 219.
2. M. A. Grusak and D. D. Penna, *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 1999, **50**, 133.
3. C. Manach, G. C. Williamson, A. Morand, Scalbert and C. Remesy, *Am. J. Clin. Nutr.*, 2005, **81**, 230S.
4. S. Kajiwara, H. Gandhi and Z. Ustunol, *J. Food Prod.*, 2002, **65**, 214.
5. W. S. Jansen, Van Rensburg, W. Van Averbeke, R. Slabbert, M. Faber and P. Van Jaarsveld, *Water, S. A.*, 2007, **33**, 317.
6. I. F. Smith, *Niger. J. Nutr. Sci.*, 1985, **4**, 21.
7. R. S. Raghuvanshi, R. Singh and R. Singh, *Int. J. Food Sci. Nut.*, 2001, **52**, 331.
8. E. M. Priya, K. M. Gothandam and S. Karthikeyan, *Am. J. Food Tech.*, 2012, **7**, 43.
9. P. Shylaja, K. M. Gothandam and S. Karthikeyan, *Res. Pharm.*, 2011, **1**, 22.
10. K. M. Gothandam, R. Aishwarya and S. Karthikeyan, *J. Phytol.*, 2010, **2**, 1.
11. A. O. Fasuyi, *Afr. J. Biotechnol.*, 2006, **5**, 49.
12. S. Saranya, S. Karthikeyan and K. M. Gothandam, *Pharmocol.*, 2010, **3**, 937.