ceramic grade kaolin from Deopani deposit of Assam. In Proc. of International Seminar on Mineral Processing Technology, MPT – 2003, Panjim, Goa, 6–8 February 2003, pp. 288–291.
18. Saikia, N. J., Bharali, D. J., Sengupta, P., Bordolo, I. D., Goswamee, R. L., Saikia, P. C. and Borthakur, P. C., Characterization, beneficiation and utilization of a kaolinite clay from Assam, India. *Appl. Clay Sci.*, 2003, **24**, 93–103.
19. Sengupta, P., Saikia, N. J., Bharali, D. J., Saikia, P. C. and Borthakur, P. C., SEM–EDXA investigation of a kaolintie clay from Deopani, Assam. In 49th Annual Technical Session of Assam Science Society, Regional Research Laboratory, Jorhat, Assam, 11 January 2004, p. 25.
20. Sengupta, P., Saikia, N. J., Bharali, D. J., Saikia, P. C. and Borthakur, P. C., Morphology of a kaolinite clay from Assam, India: an investigation by SEM–EDXA (communicated), 2004.
21. Goodman, B. A. and Hall, P. L., Electron paramagnetic resonance spectroscopy. In *Clay Mineralogy: Spectroscopic and Chemical Determinative Methods* (eds Wilson, M. J.), Chapman & Hall, London, 1994, 1st edn, p. 173; 201–209.
22. IS: 2840, Indian standard specification, Indian standard method of mixing. Indian Standard Institution, New Delhi, 1965.
23. Jones, J. P. E., Angel, B. R. and Hall, P. L., Electron spin resonance studies of doped synthetic kaolinite. II. *Clay Miner.*, 1974, **10**, 257–269.
24. Manhaes, R. S. T. *et al.*, Soil characterization using X-ray diffraction, photoacoustic spectroscopy and electron paramagnetic resonance. *Appl. Clay Sci.*, 2002, **21**, 303–311.
25. Schellmann, W., Die bildungsbedingungen sedimentärer chamosit- und hämatiteisenerze am beispiel der lagerstätte echte. *Neues Jahrb. Mineral., Abh.*, 1969, **111**, 1–31.
26. Maynard, J. B., In *Geochemistry of Sedimentary Ore Deposits*, Springer-Verlag, New York, 1983, pp. 40–45.
27. NBSS and LUP: Bulletin 66, *Soils of Assam for Optimizing Land Use.* Soils of India Series, National Bureau of Soil Survey and Land Use Planning, Nagpur, 1999, pp. 14–15.
28. Russell, J. D. and Fraser, A. R., Infrared methods. In *Clay Mineralogy: Spectroscopic and Chemical Determinative Methods* (ed. Wilson, M. J.), Chapman & Hall, London, 1994, 1st edn, pp. 11–67.
29. Wells, M. A., Gilkes, R. J. and Fitzpatrick, R. W., Properties and acid dissolution of metal-substituted hematites. *Clays Clay Miner.*, 2001, **49**, 60–72.

ACKNOWLEDGEMENTS. We are grateful to the Ministry of Coal and Mines, New Delhi for financial grant and the State Directorate of Geology and Mining, Government of Assam, Guwahati, for providing the investigated kaolin sample. We are also grateful to Dr P. Kotoky, Scientist, RRL, Jorhat for helpful discussion and Dr P. G. Rao, Director, RRL, Jorhat for his encouragement in the work.

Received 3 November 2005; revised accepted 28 February 2006

# Antioxidant activity of *Garcinia indica* (kokam) and its syrup

**Akanksha Mishra[1], Mrinal M. Bapat[1], Jai C. Tilak[2] and Thomas P. A. Devasagayam[2,*]**

[1]Department of Biochemistry, The Institute of Science, Madam Cama Road, Mumbai 400 032, India
[2]Radiation Biology and Health Sciences Division, Bhabha Atomic Research Centre, Mumbai 400 085, India

***Garcinia indica*** **(kokam) is an Indian spice, the fruit rind of which is used in cooking, cosmetics and has several medicinal properties. Its syrup is consumed as a soft drink during summer. We have examined the antioxidant activity of aqueous and boiled extracts corresponding to their use in cooking and home remedies, besides the commercial kokam syrup. The assays employed are ORAC, FRAP, ABTS and the ability to inhibit lipid peroxidation in rat liver mitochondria. Kokam syrup and the two aqueous extracts had significant antioxidant effects in the above assays. They have high ORAC values (29.3, 24.5 and 20.3), higher than those reported for other spices, fruits and vegetables. The high antioxidant activity of kokam adds one more positive attribute to its known medicinal properties and hence its use in cooking, home-remedies and as a soft drink may be promoted.**

**Keywords:** Antioxidant activity, kokam, medicinal properties, syrup.

ANTIOXIDANTS are micronutrients that have gained importance in recent years due to their ability to neutralize free radicals or their actions[1]. Free radicals have been implicated in the etiology of several major human ailments, including cancer, cardiovascular diseases, neural disorders, diabetes and arthritis[2–4]. Due to the recent trends in nutrition towards development of healthy foods in the form of 'functional foods', one of the desirable properties in a dietary component is considered to be its antioxidant effect[5,6].

*Garcinia indica* (dried rind known as 'kokam') is an Indian spice used in many parts of the country for making several vegetarian and non-vegetarian 'curry' preparations, including the popular 'solkadhi'. The fruits are steeped in sugar syrup to make 'amrutkokam', a healthy soft drink to relieve sunstroke, which is popular during summer[5,7,8]. It is a traditional home remedy in case of flatulence, heat strokes and infections[7]. Many therapeutic effects of the fruit have been described in traditional medicine based on Ayurveda. These include its usefulness as an infusion, in skin ailments such as rashes caused by allergies; treatment of burns, scalds and chaffed skin; to relieve sunstroke; remedy for dysentery and mucous diarrhoea; an appetizer and a good liver tonic; to improve appetite and to allay thirst;

*For correspondence. (e-mail: tpad@apsara.barc.ernet.in)

as a cardiotonic and for bleeding, piles, dysentery, tumours and heart diseases.

One of the ingredients of kokam, hydroxycitric acid (HCA), has been patented for use as an hypocholesterolaemic agent. HCA is a potential anti-obesity agent[9]. It suppresses fatty acid synthesis, lipogenesis, food intake and induces weight loss[9]. Garcinol, a polyisoprenylated benzophenone purified from *G. indica* fruit rind, displays antioxidant, anti-cancer and anti-ulcer properties[10,11]. Apart from HCA and garcinol, kokam contains other compounds with potential antioxidant properties. These include citric acid, malic acid, polyphenols, carbohydrates[1,3], anthocyanin pigments and ascorbic acid[5].

Though studies have been carried out on the antioxidant activities of these two isolated components from *G. indica*, besides an organic extract, the antioxidant activity of the rind and its various preparations as is being used in cooking, in soft drinks and medicinal preparations has not been evaluated. The levels of antioxidant action studied are (i) radical formation by Ferric Reducing/Antioxidant Power (FRAP); (ii) radical scavenging by Oxygen Radical Absorbance Capacity (ORAC) and 2,2′-azobis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), and (iii) prevention of membrane damage as measured by lipid peroxidation. These are standard assays used for determining the antioxidant abilities of food preparations.

The aqueous and boiled extracts were prepared so as to simulate conditions of their extraction pertaining to their use for cooking purposes. The aqueous extract was prepared by adding the crushed rind to distilled water and stirring for 60 min, similar to use in many curry preparations. For boiled water extract, the crushed rind was boiled in distilled water (DW) for 30 min, similar to its use in curry preparations. Commercially available kokam syrup was also used for assessing its antioxidant effects by diluting it to 25% (v/v) concentration using DW as is generally consumed as ‘kokam squash’.

In FRAP assay, the FRAP reagent is prepared by adding 2,4,6-tripyridyl-*s*-triazine (TPTZ) and ferric chloride, forming the $Fe^{3+}$–TPTZ complex. This is reduced to $Fe^{2+}$–TPTZ, at low pH, when an antioxidant is present, which has an intense blue colour with absorption maximum[12] at 595 nm. The calibration curve was plotted with OD versus concentration of $FeSO_4$ in the range of 0–1 mM. The results are expressed as Ascorbic acid Equivalent Antioxidant Capacity (AEAC) in terms of mM. In ferrylmyoglobin/$ABTS^+$ assay, the inhibition of radical formation by the extracts was determined[13]. The calibration curve was plotted with lag time in seconds versus concentration of the standard antioxidants (L-ascorbic acid). The results are expressed as AEAC in terms of mM. In ORAC assay, oxygen radical absorbance capacity was measured by detection of 2.2′-azobis (2-amidinopropane) dihydrochloride (AAPH)-induced chemical damage to β-phycoerythrin through decrease in fluorescence emission. Fluorescence was recorded every 5 min till the last reading is less than 5% of the first reading. ORAC values were calculated in terms of micromoles of trolox equivalent/g of fresh weight[14]. This is considered as a ‘standard’ assay, especially while comparing different food preparations.

Three-months-old female Wistar rats (weighing about 250 ± 20 g) were used in the preparation of mitochondria. Mitochondria from rat liver was isolated as described earlier[15]. Protein was estimated[16] and pellets suspended in the 0.15 M Tris-HCl buffer, pH 7.4 at a concentration of 10 mg protein/ml. Oxidative damage was induced by ascorbate–$Fe^{2+}$ system[17]. Thiobarbituric acid reactive substances formed were estimated spectrophotometrically (532 nm)[17], as malondialdehyde equivalents after accounting for appropriate blanks. Malondialdehyde standard was prepared by the acid hydrolysis of tetraethoxypropane.

Table 1 presents data on AEAC values for the extracts and syrup with the FRAP and ferrylmyoglobin/ABTS assays at low concentration of 0.1%, besides values of ORAC assay. In FRAP assay, boiled aqueous kokam extract possessed the highest AEAC value of 0.057 mM, followed by the syrup and aqueous extract with AEAC of 0.049 and 0.047 mM, respectively. In case of ferrylmyoglobin/ABTS assay, among all kokam extracts, kokam syrup was the most potent in the inhibition of ABTS radical formation with AEAC of 1.005 mM, followed by aqueous extract and boiled aqueous extract with AEAC of 0.754 and 1.19 mM respectively.

The aqueous extracts of *G. indica* showed significant values by ORAC assay having 29.3, 24.5 and 20.3 μmol of Trolox equivalent/g fresh weight values for aqueous, boiled aqueous extracts and kokam syrup respectively. The data reveal that among the kokam extracts, the aqueous extract possessed the highest antioxidant potential.

Table 2 shows data on lipid peroxidation in rat liver mitochondria on ascorbate–$Fe^{2+}$-induced oxidative damage. Among the kokam extracts, kokam syrup (final concentration 2.5%) was the most effective giving 78% protection against lipid peroxidation, followed by 0.1% aqueous and boiled aqueous extracts with 40 and 51% protection, respectively.

Aqueous kokam extract and kokam syrup exhibit inhibition of peroxyl radical-induced phycoerythrin oxidation in ORAC assay. Our results show that the values of kokam extract ranging from 20 to 29 are higher than those observed for some fruits and vegetables, including those for garlic (19.4), blueberry (15.9), strawberry (15.36), aqueous turmeric extracts (13.49), spinach (12.6), plum (9.49), beets (8.4), orange (7.5), onion (4.5), cauliflower (3.8), cabbage (3.0), banana (2.21) and carrot (2.10)[18–20]. It is worth noting that the ORAC values of kokam and its syrup are higher than those reported for fruits used for syrups such as strawberry, plum and orange. Hence kokam syrup may be considered healthier than juice prepared from other fruits due to its higher antioxidant availability. In case of FRAP assay, the values for kokam extract are found to be lesser than

**Table 1.** Antioxidant measurement of *Garcinia indica* extracts and kokum syrup

| *Garcinia indica* extract | FRAP assay (AEAC, mM) | Ferrylmyoglobin/ ABTS assay (AEAC, mM) | ORAC assay ($\mu$mol of TE/g of fresh wt) |
|---|---|---|---|
| Aqueous extract | 0.047 ± 0.002 | 1.005 ± 0.08 | 29.3 ± 0.80 |
| Boiled aqueous extract | 0.057 ± 0.001 | 0.754 ± 0.12 | 24.5 ± 0.43 |
| Kokum syrup | 0.049 ± 0.001 | 1.19 ± 0.06 | 20.3 ± 0.08 |

Data expressed as mean ± SE of four individual experiments for all three assays. Data expressed as micromoles of Trolox equivalents per g of fresh weight for ORAC assay. Data expressed as TAA as% ascorbic acid equivalent measured. Ascorbic acid equivalent antioxidant capacity (AEAC, mM).

**Table 2.** Effect of *G. indica* extracts and kokum syrup on ascorbate–$Fe^{2+}$-induced lipid peroxidation in rat liver mitochondria

| Condition | nmol TBARS/mg protein | Per cent inhibition |
|---|---|---|
| Control (untreated) | 4.83 ± 0.20 | – |
| Damage (treated with ascorbate-$Fe^{2+}$) | 30.94 ± 0.51 | – |
| Aqueous extract (0.1%) | 20.62 ± 0.49 | 39.5 |
| Boiled aqueous extract (0.1%) | 17.76 ± 0.05 | 50.5 |
| Kokum syrup (2.5%) | 10.56 ± 0.98 | 78.1 |

Values are mean ± SE from four individual experiments. TBARS, Thiobarbituric acid reactive substances.

those of turmeric and other medicinal preparations, whereas in ferrylmyoglobin/ABTS assay, kokam extract and kokam syrup possessed higher antioxidant capacity than turmeric extract[20]. The mechanisms involved in many human diseases such as hepatotoxicities, hepatocarcinogenesis, diabetes, malaria, acute myocardial infarction, skin cancer include free radicals-induced lipid peroxidation as the main source of membrane damage[3]. The antioxidant action can also be measured by inhibition of damage caused by free radicals[19,21]. In our studies, ascorbate–$Fe^{2+}$, a system relevant to endogenous oxidative damage was used for rat liver mitochondria.

Modulation of diseased states such as cardiovascular ailments, neurological disorders, cancer and diabetes using dietary components, including fruits and vegetables, natural products and medicinal plants as a possible therapeutic measure has become a subject of active scientific investigations[22,23]. The very concept of food is changing from a past emphasis on health maintenance to the promising use of food to promote better health to prevent chronic illnesses. The advent of functional foods may allow us to improve public health. ‘Functional foods’ are those that provide more than simple nutrition; they supply additional physiological benefits to the consumer[24,25]. In India also, the demand for functional foods is increasing in recent years. In this aspect, foods rich in preparations from kokam and its syrup can be considered as functional foods.

The assays used for testing the antioxidant capacities of kokam extract act at various levels of antioxidant activity at which the damage caused by free radicals can be prevented. Thus these experiments could be useful in detecting the mechanism of prevention of free-radical damage by various preparations of kokam. Our study reveals that kokam has significant antioxidant effects acting at different levels. Its antioxidant activity is higher than that reported for many fruits and vegetables. Besides, it is cheap, readily available to all strata of society, with medicinal properties attributed to it. At present, the use of kokam and its syrup is mainly restricted to certain states on the west coast of India. Due to its health benefits, it needs to be promoted.

In conclusion, our studies using biochemical assays pertaining to different levels of antioxidant action, show that various preparations of kokam have significant antioxidant activities.

1. Cadenas, E. and Packer, L. (eds), *Hand Book of Antioxidants*, Plenum, New York, 1996.
2. Sies, H. (ed.), *Antioxidants in Disease, Mechanisms and Therapy*, Academic Press, New York, 1996.
3. Yoshikawa, T. *et al.* (eds), *Free Radicals in Chemistry, Biology and Medicine*, OICA International, London, 2000.
4. Devasagayam, T. P. A., Tilak, J. C., Boloor, K. K., Sane, K. S., Ghaskadbi, S. and Lele, R. D., Free radicals and antioxidants in human health: current status and future prospects. *J. Assoc. Physicians India*, 2004, 794–804.
5. Peter, K. V., *Handbook of Herbs and Spices*, CRC Press, Boca Raton, FL, USA, 2001.
6. Wildman, R. E. C., *Handbook of Neutraceuticals and Functional Foods*, CRC Press, Boca Raton, FL, USA, 2001.
7. Kirtikar, K. R. and Basu, B. D., *Indian Medicinal Plants, Vol. I*, (eds Blatter, E. *et al.*), Allahabad, India, 1984.
8. Drury, H., *The Useful Plants of India*, Allied Book Centre, Dehradun, 1991.
9. Jena, B. S., Jayaprakasha, G. K., Singh, R. P. and Sakariah, K. K., Chemistry and biochemistry of (–)-hydroxycitric acid from *Garcinia*. *J. Agric. Food Chem.*, 2002, **50**, 10–22.

10. Yamaguchi, F., Ariga, T., Yoshimura, Y. and Nakazawa, H., Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. *J. Agric. Food Chem.*, 2000, **48**, 180–185.
11. Selvi, A. T., Joseph, G. S. and Jayaprakasha, G. K., Inhibition of growth and aflatoxin production in *Aspergillus flavus* by *Garcinia indica* extract and its antioxidant activity. *Food Microbiol.*, 2003, **20**, 455–460.
12. Benzie, I. F. and Strain, J. J., The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': the FRAP assay. *Anal. Biochem.*, 1996, **239**, 70–76.
13. Rice-Evans, C. and Miller, N., Total antioxidant status in plasma and body fluids. *Methods Enzymol.*, 1994, **234**, 279–293.
14. Cao, G. and Prior, R. L., Measurement of oxygen radical absorbance capacity in biological samples. *Methods Enzymol.*, 2002, **299**, 50–62.
15. Lakshmi, B., Tilak, J. C., Adhikari, S., Devasagayam, T. P. A. and Janardhanan, K. K., Inhibition of lipid peroxidation induced by γ-radiation and AAPH in rat liver and brain mitochondria by selected mushrooms. *Curr. Sci.*, 2005, **88**, 484–488.
16. Lowry, O. H., Rosebrough, N. J., Farr, L. A. and Randal, R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 1951, **193**, 265–267.
17. Devasagayam, T. P. A. and Pushpendran, C. K., Changes in ascorbate-induced lipid peroxidation of hepatic rough and smooth microsomes during postnatal development and ageing of rats. *Mech. Ageing Dev.*, 1986, **34**, 13–22.
18. Cao, G., Sofic, E. and Prior, R. L., Antioxidant capacity of tea and common vegetables. *J. Agric. Food Chem.*, 1996, **44**, 3426–3431.
19. Lachnicht, D., Brevard, P. B., Wagner, T. L. and DeMars, C. E., Dietary oxygen radical absorbance capacity as a predictor of bone mineral density. *Nutr. Res.*, 2002, **22**, 1389–1399.
20. Tilak, J. C., Banerjee, M., Hari Mohan and Devasagayam, T. P. A., Antioxidant availability of turmeric in relation to its medicinal and culinary uses. *Phytother. Res.*, 2004, **18**, 798–804.
21. Devasagayam, T. P. A., Boloor, K. K. and Ramasarma, T., Methods for estimating lipid peroxidation: Analysis of merits and demerits. *Indian J. Biochem. Biophys.*, 2003, **40**, 300–308.
22. Hertog, M. G. L., Feskens, E. J. M., Hollman, P. C. H., Katan, M. B. and Kromhout, D., Dietary antioxidant flavonoids and risk of coronary heart disease: The Zutphen elderly study. *Lancet*, 1993, **342**, 1007–1011.
23. Surh, Y.-J., Cancer chemoprevention with dietary phytochemicals. *Nature Rev. Cancer*, 2003, **3**, 768–780.
24. Krishnaswamy, K., Indian functional foods: Role in prevention of cancer. *Nutr. Rev.*, 1996, **54**, S127–S131.
25. Devasagayam, T. P. A., Tilak, J. C. and Singhal, R., Functional foods in India: history and scope. In *Angiogenesis, Functional and Medicinal Foods* (eds Losso, J. N. *et al.*), Marcel Dekker Inc, New York (in press).

ACKNOWLEDGEMENTS. We thank Dr T. K. Dongre for suggestions and Dr K. K. Boloor and Mrs Jayashree K. Boloor for help.

Received 16 June 2005; revised accepted 8 March 2006

# Enhanced genetic transformation efficiency of mungbean by use of primary leaf explants

**L. Sita Mahalakshmi, T. Leela, S. Manoj Kumar, B. Kiran Kumar, B. Naresh and Prathibha Devi***

Department of Botany, Osmania University, Hyderabad 500 007, India

**Transgenic mungbean plants [*Vigna radiata* (L.) Wilczek] were developed via genetic transformation of primary leaf explants with disarmed *Agrobacterium tumefaciens* strain C-58 harbouring a binary plasmid, pCAMBIA–1301 [(containing genes for β-glucuronidase (*GUS*) and hygromycin phosphotransferase (*hpt*)]. A genotype independent, high frequency plant regeneration protocol was initially developed with a survival of about 90% in two cultivars of mungbean by use of primary leaf explants (cut at the node) from four-day-old and ten-day-old seedlings. The co-cultivated explants (taken from ten-day-old seedlings of the cultivar K-851) were cultured on hygromycin selection to recover putatively transformed plants, which were acclimatized and moved to the glasshouse. The time required for regeneration of transgenic plantlets from the transformed explants was nearly 12 weeks. The transformed plants were morphologically similar to the seed-germinated plants. Transformation was confirmed by histochemical assay for *GUS* activity. Stable integration of the marker gene in the T0 transgenics and its inheritance in the T1 transgenics has been confirmed through molecular analysis. The enhanced genetic transformation efficiency obtained presently is repeatable and can be used to mobilize genes of agronomic importance into elite cultivars of mungbean.**

**Keywords:** *Agrobacterium*, enhanced genetic transformation, mungbean, primary leaf explants.

GENE transfer to plants has opened new ways for the use of recombinant DNA technology and is useful in complementing the conventional breeding programmes[1,2].

Mungbean [*Vigna radiata* (L.) Wilczek] ($2n = 22$) is an important foodgrain legume crop of Asia and a good source of dietary protein. It is cultivated in India, South America and Australia. However, its production is limited due to certain undesirable agronomic traits and its susceptibility to biotic stresses like diseases caused by fungi, bacteria, viruses and insect pests. Genetic improvement of mungbean is possible by transfer of agronomically important genes through genetic transformation.

Although efficient plant regeneration and genetic transformation protocols have been developed in some legumes[1,3–18], lack of an efficient regeneration system has limited the improvement of mungbean via genetic trans-

*For correspondence. (e-mail: prathibhadevi@osmania.ac.in)