



Total phenolic content and antioxidative potential of amla and its baked products

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Abstract: In the present study, value added baked products from fresh and powdered amla (*Phyllanthus emblica*) were developed and were evaluated for their organoleptic acceptability, total phenol content and antioxidative potential. Varieties used for the study were 'NA-7' and 'Francis'. Amla incorporation at a level ranging between 5 to 30% was found to be highly acceptable organoleptically ($p > 0.01$) on evaluation of fresh amla cake, amla-date cake, amla-apple pie, amla-jam cookies, amla powder cake and amla salty biscuits. Total phenol content of amla pulp, powder, fresh amla cake and amla powder cake ranged between 0.95 to 68 mg/g, the highest being in 'Francis' powder and lowest in fresh amla cake. *In vitro* assay systems were used for evaluating the antioxidative potential using methanol as a solvent. Maximum hydroxyl radical scavenging potential was exhibited by Francis powder (EC_{50} eq/g=3200) followed by 'NA-7' powder (EC_{50} eq/g=1818.2) and fresh amla cake (EC_{50} eq/g=154.3). In case of nitric oxide radical, inhibition was shown by pulp only, 'Francis' pulp being a better scavenger (EC_{50} eq/g=869.5) than 'NA-7' (EC_{50} eq/g=689.6). Highest superoxide radical scavenging potential was shown by 'Francis' pulp (EC_{50} eq/g=2000) followed by 'Francis' powder (EC_{50} eq/g= 1538.5) and 'NA-7' pulp (EC_{50} eq/g=1428.6). Thus, 'Francis' was found to be superior than 'NA-7' in exhibiting antioxidative potential and maximum free radical inhibition in terms of EC_{50} eq/g was shown by powder followed by pulp.

Key words: Amla, Baked products, Total phenols, Hydroxyl radical, Nitric oxide radical, Superoxide radical

Introduction

Amla (*Phyllanthus emblica*) a moderately sized deciduous tree is quite a hardy plant, a prolific bearer and highly remunerative even without much care. The fruit may be stored in cold storage for 7-8 days at 32-35° F. Amla fruits are hard and unyielding to touch and as such well suited for long distance transportations. The fruit is a rich source of ascorbic acid. It also contains tannins and many polyphenolic compounds. Amla is a rare example of an edible material which is rich in tannins as well as ascorbic acid. The astringency of the fruit may be assigned to polyphenols present in it. It is probably these polyphenols which are responsible for stability of the ascorbic acid content in amla. Being a rich source of polyphenols and ascorbic acid, amla exhibits strong antioxidative potential which helps to boost the functioning of immune system.

Oxidative stress is found to play an important contributory role in pathogenesis of ageing, inflammation and cancer. Free radicals have also been implicated as playing a role in etiology of various cardiovascular diseases, neurodegenerative chronic inflammatory diseases such as atherosclerosis, asthma, stroke, vasospasms and liver damage (Allen and Tresini, 2000). In our body, protection against free radicals is provided either by the antioxidant enzymes which include glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase or the non-enzymatic antioxidants supplied through our diet which include glutathione, thiols, vitamin C, vitamin E, vitamin A, some metals and polyphenols like isoflavones, gallic acid, kaempferol etc.

It is now widely held that the antioxidants contained in fruits and vegetables can provide protection against certain human

degenerative conditions associated with free radical damage. Polyphenols contribute substantially to the antioxidant complement of many small fruit species (Kalt *et al.*, 2001). Natural antioxidants have gained considerable interest in recent years for their role in prevention of auto-oxidation of fats, oils and fat containing food products.

Fruits containing high levels of phenolic antioxidants would be attractive to health conscious consumers, therefore optimization of production and processing factors affecting antioxidant capacity is desirable. Amla is one of the cheapest fruit with a strong antioxidative potential but its economic utilization is still lagging due to its restricted availability from mid November to third week of January. In order to utilize the immense nutritive and antioxidative potential of amla, it is essential to process it into powder and develop value added products. Keeping in view the protective effects of amla against free radicals and the paucity of data regarding the incorporation of amla in baked products, the present study has been designed to develop value added baked products from fresh and powdered amla and to evaluate their total phenolic content and antioxidant activity.

Materials and Methods

Procurement of varieties: 'NA-7' and 'Francis' varieties of amla were procured from Department of Horticulture, Punjab Agricultural University and half of them were processed into powder by cabinet drying and then grinding in a mixer.

Development of baked products: Fresh amla pieces, pulp and amla powder of the variety showing higher ascorbic acid content were incorporated in baked products like fresh amla cake, amla-

date cake, amla-apple pie, amla-jam cookies, amla powder cake and amla salty biscuits.

Organoleptic evaluation and standardization of baked products: The amla incorporated baked products were organoleptically evaluated for their consumer acceptance by a panel of 8 judges on a nine point hedonic scale given by Ranganna (1986). The products were then standardized with the maximum acceptable levels.

Preparation of extracts: Extract preparation of amla pulp, powder, fresh amla cake and amla powder cake was done using methanol as a solvent for evaluation of total phenolic content and antioxidant activity.

Amla pulp: After squeezing out juice and drying, 5 g pulp was added to 50 ml of methanol and kept overnight. It was then filtered through G2 funnel and the volume was made 100 ml.

Amla powder: 5 g powder was added to 50 ml methanol and kept overnight. It was then filtered through G2 funnel and the volume was made 100 ml. 10 ml was drawn from it and kept in oven for drying at 37°C. After drying weight was recorded and further dilution was done to set the conc. of 1 mg ml⁻¹.

Amla cakes: 10 g homogenously blended sample of cake was added to 25 ml methanol and kept overnight. It was then filtered through G2 funnel and the volume was made 50 ml. 10 ml was drawn from it and kept in oven for drying at 37°C. After drying, weight was recorded and further dilution was done to set the conc. of 10 mg ml⁻¹.

Estimation of total phenols: Total phenolic content in amla pulp, powder and two commonly consumed baked products- fresh amla cake and amla powder cake was estimated by the method given by Swain and Hillis (1959) using Folin phenol reagent and the blue colour developed was read after 1 hour at 760 nm against the blank. The conc. of total phenols was read from the standard curve (Fig. 1) prepared by using gallic acid (10-50 mg range).

Antioxidant activity: Hydroxyl radical, nitric oxide radical and superoxide radical scavenging potential of amla pulp, amla powder, fresh amla cake and amla powder cake were evaluated using *in vitro* models. The free radical scavenging potential was denoted in terms of EC₅₀ equivalents/g. EC₅₀ indicates the concentration of sample at which 50% inhibition of free radicals take place. By using EC₅₀ values, EC₅₀ equivalents were calculated taking dilution factor into consideration.

Evaluation of hydroxyl radical scavenging potential using *in vitro* model: In order to evaluate hydroxyl radical scavenging potential of methanolic extracts of samples, the inhibition of deoxyribose degradation by hydroxyl radical generated from Fe²⁺-ascorbate-EDTA-H₂O₂ system was determined. Deoxyribose degradation was measured as thio-barbituric acid reactive substances (TBARS) by the method of Ohkawa *et al.* (1979). The

percentage inhibition was determined by comparing the absorbance values of test and control at 532 nm.

Evaluation of nitric oxide radical scavenging activity using *in vitro* model: In order to evaluate the nitric oxide radical scavenging potential, the inhibition of chromophore formation with Griess reagent by nitric oxide radical spontaneously generated from sodium nitroprusside was determined by the method of Green *et al.* (1982). The absorbance of chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling to naphthyl ethylene diamine dihydrochloride was read at 546 nm. The percentage inhibition of nitric oxide generation was measured by comparing the absorbance value of test and control.

Evaluation of superoxide radical scavenging potential using *in vitro* model: In order to evaluate superoxide radical scavenging potential, the inhibition of Nitro blue tetrazolium (NBT) reduction by superoxide radical generated from photo reduction of riboflavin was determined by the method of Mc Cord and Fridovich (1969). The optical density was recorded at 530 nm. The percentage inhibition of superoxide generation was measured by comparing the absorbance values of test and control compounds.

Results and Discussion

Organoleptic evaluation of amla incorporated baked products: Organoleptic evaluation of amla incorporated baked products resulted in 30% level of acceptance for fresh amla cake, amla-date cake, amla-apple pie, amla-jam cookies and 8% and 5% level of acceptance for amla powder cake and amla salty biscuits respectively.

Total phenolic content: Table 1 shows the total phenolic content of amla pulp, powder, fresh amla cake and amla powder cake. Among the two varieties, *Francis* had a higher total phenol content as compared to *NA-7*. *Francis* and *NA-7* pulp had a total phenol content of 11 mg g⁻¹ of pulp and 10 mg g⁻¹ of pulp respectively. *Francis* and *NA-7* powder had a total phenol content of 68 mg g⁻¹ and 64 mg g⁻¹ respectively. Kaur and Kapoor (2002) reported that amla had more than 70% antioxidant activity which correlated significantly and positively with total phenolics ($r = 0.6578$, $p < 0.05$). Kumar *et al.* (2006) reported that high level of antioxidant activity in *E. officinalis* has been attributed to the phenolic content (12.9%, w/w, correlation coefficient $r = 0.74$) in them. In many fruit crops, antioxidant activity (measured as oxygen radical absorbing capacity ORAC) is positively correlated with their content of anthocyanins and total phenolics. Genera, species and genotypes vary with respect to phenolic content (Kalt *et al.*, 2001). Fresh amla cake had a total phenolic content of 0.95 mg g⁻¹ and that of amla powder cake was 1.10 mg g⁻¹. Control cake had a total phenolic content of 0.645 mg g⁻¹.

Antioxidant activity: Hydroxyl radical, nitric oxide radical and superoxide radical scavenging potential of amla juice, amla powder and two commonly consumed baked products- fresh amla cake and amla powder cake were evaluated using *in vitro* models. The

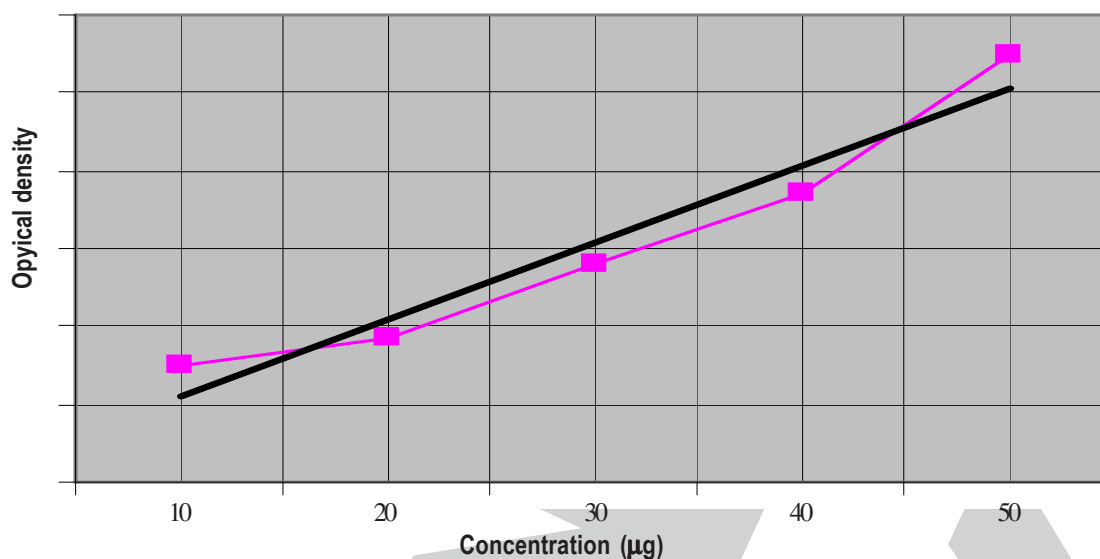


Fig. 1: Standard curve for gallic acid

free radical scavenging potential was denoted in terms of EC_{50} equivalents/g. EC_{50} indicates the concentration of sample at which 50% inhibition of free radicals take place. By using EC_{50} values, EC_{50} equivalents were calculated taking dilution factor into consideration.

Hydroxyl radical scavenging potential: Table 2 shows the EC_{50} values and EC_{50} equivalents/g of samples. The concentrations of 'NA-7' and 'Francis' pulp needed for 50% inhibition (EC_{50}) of hydroxyl radicals were found to be 200 mg and 157.5 mg respectively. EC_{50} equivalents/g of amla pulp for hydroxyl radical scavenging potential were found to be 100 and 127 for 'NA-7' and 'Francis' respectively. Sabu and Kuttan (2002) reported that the concentration of methanolic extract of *Embllica officinalis* needed for 50% inhibition of hydroxyl radical was 155.5 mg ml⁻¹ which supports the present finding of *Francis* pulp, inhibiting 50% of hydroxyl radicals at 157.5 mg ml⁻¹.

Concentrations of 'NA7' variety and 'Francis' variety of amla powder needed for 50% inhibition (EC_{50}) were found to be 110 mg and 50 mg respectively. The EC_{50} equivalents g⁻¹ of amla powder for hydroxyl radical scavenging potential were found to be 1818.2 and 3200 for 'NA-7' and 'Francis' variety respectively.

Among the cakes prepared from fresh amla and its powder, the methanolic extract of fresh amla incorporated cake showed better inhibition of hydroxyl radical. The EC_{50} values were 87.5mg, 100mg and 165mg for fresh amla incorporated cake, amla powder incorporated cake and control cake respectively. The EC_{50} equivalents g⁻¹ of cakes were 154.3, 105 and 60.6 respectively. The results indicate that the retention of EC_{50} equivalents g⁻¹ in fresh amla incorporated cake was higher than the retention in amla powder incorporated cake. The control cake also exhibited hydroxyl radical scavenging potential which might be due to the other ingredients. Among the two varieties, 'Francis' gave better results in inhibiting

hydroxyl radical than 'NA-7' variety which might be due to its higher phenolic content.

Among the several free radicals, hydroxyl radical (OH) is the most potent oxidant, produced during radiation exposure as well as Fenton reaction (Halliwell and Gutteridge, 1993; Naik et al., 2006).

Nitric oxide radical scavenging potential: Table 3 indicates the EC_{50} values and EC_{50} equivalents/g of samples. Inhibition of nitric oxide radical generation by pulp of both varieties of amla indicated a better activity of *Francis* with EC_{50} at 23 mg whereas 50% inhibition by *NA-7* was at a concentration of 29 mg. The EC_{50} equivalents/g of pulp worked out to be 869.5 and 689.6 respectively for *Francis* and *NA-7* variety.

Inhibition potential of amla powder against nitric oxide radical generation proved inefficient. The methanolic extracts of cakes did not exhibit even 50% inhibition of nitric oxide radicals.

The methanolic extracts of cakes did not inhibit even 50% of nitric oxide radicals. *Francis* variety revealed better inhibitory potential for this assay system also, indicating superiority over *NA-7* variety. Methanolic extract of amla powder and amla cakes could not inhibit even 50% of nitric oxide radicals which means the compounds responsible for contributing antioxidant activity in methanol were ineffective in scavenging nitric oxide radicals.

Superoxide radical scavenging potential: Table 4 depicts the EC_{50} values and EC_{50} equivalents/g of samples. Concentrations of 'NA-7' and 'Francis' pulp needed for 50% inhibition (EC_{50}) of superoxide radicals were found to be 14 and 10 mg respectively. The EC_{50} equivalents/ml were 1428.6 and 2000 for 'NA-7' and 'Francis' respectively. This finding is close to the investigation carried by Sabu and Kuttan (2002) who reported that 6.5 mg ml⁻¹ of methanolic extract of *Embllica officinalis* was required to inhibit 50% superoxide radicals.

Table - 1: Total phenolic content of amla pulp, powder and cake (mg g⁻¹)

Type	Sample	Total phenols
Fresh	NA-7 pulp	10.0
	Francis pulp	11.0
Powder	NA-7 powder	64.0
	Francis powder	68.0
Product	Fresh amla cake	0.95
	Amla powder cake	1.10
	Control cake	0.65

Table - 2: EC₅₀ equivalents /g of sample for hydroxyl radical (methanolic extracts)

Sample	EC ₅₀	EC ₅₀ equivalents/g
NA-7 pulp	200 mg	100
Francis pulp	157.5 mg	127
NA-7 powder	110 mg	1818.2
Francis powder	50 mg	3200
Fresh amla cake	87.5 mg	154.3
Amla powder cake	100 mg	105
Control cake	165 mg	60.6

Table - 3: EC₅₀ equivalents /g of sample for nitric oxide radical (methanolic extracts)

Sample	EC ₅₀	EC ₅₀ equivalents/g
NA-7 pulp	29 mg	689.6
Francis pulp	23 mg	869.5
NA-7 powder	-	-
Francis powder	-	-
Fresh amla cake	-	-
Amla powder cake	-	-
Control cake	-	-

Table - 4: EC₅₀ equivalents/g of sample for superoxide radical (methanolic extracts)

Sample	EC ₅₀	EC ₅₀ equivalents/g
NA-7 pulp	14 mg	1428.6
Francis pulp	10 mg	2000
NA-7 powder	39 mg	820.5
Francis powder	26 mg	1538.5
Fresh amla cake	69 mg	195.6
Amla powder cake	32.5 mg	323.1
Control cake	115 mg	86.95

Methanolic extract of powders of both the varieties also showed significant superoxide radical scavenging potential, NA-7 with EC₅₀ at 39 mg. and Francis with EC₅₀ at 26 g. and their EC₅₀ equivalents/g were 820.5 for NA-7 and 1538.5 for Francis.

Among the cakes, amla powder incorporated cake exhibited best activity with EC₅₀ at 32.5 mg followed by fresh amla incorporated cake with EC₅₀ at 69 mg and lastly control cake with EC₅₀ at 115 mg. Their EC₅₀ equivalents/g were 323.1, 195.6 and 86.95 for amla

power incorporated cake, fresh amla incorporated cake and control cake respectively.

Conclusion

Organoleptic evaluation of baked products of amla and its powder was found to be highly acceptable ($p < 0.01$) with 30% level of acceptance for fresh amla cake, amla-date cake, amla-apple pie, amla-jam cookies and 8 and 5% level of acceptance for amla powder cake and amla salty biscuits respectively. The highest total phenolic content was found in amla powder followed by pulp and amla cakes. Among the two varieties Francis variety had higher total phenolic content than NA-7 variety. Among the cakes, amla powder cake had higher total phenolic content than fresh amla cake. The *in vitro* assay systems used for evaluating antioxidative potential revealed the superiority of 'Francis' over 'NA-7' among the two varieties. Francis powder exhibited highest hydroxyl radical scavenging potential and 'Francis' pulp exhibited highest nitric oxide and superoxide radical scavenging potential. The antioxidative potential in terms of EC₅₀ eq/g was highest for 'Francis' powder. Thus, amla powder can be utilized in the diet as an ingredient in recipes because of its longer shelf life and higher antioxidant activity which may be attributed to its higher phenolic content. Being natural and safe, it can replace synthetic antioxidants used in food industries. In the present investigation, *in vitro* assay systems were used for determining antioxidant activity of amla and its baked products. Further, *ex-vivo* and *in vivo* studies are required to know the accurate beneficial effects of amla incorporated products on human body.

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