WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 3, 910-923 Research Article ISSN 2278 – 4357

UTILIZATION OF MATURE AND IMMATURE LEAVES OF MANGO (*MANGIFERA INDICA*) FOR PREPARATION AND PHARMACOGNOSTIC EVALUATION OF JELLIES

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Article Received on
08 Jan 2019,

Revised on 29 Jan. 2019,
Accepted on 18 Feb. 2019

DOI: 10.20959/wjpps20193-13255

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ABSTRACT

A method for preparation of jellies fortified with biomolecules from immature and mature mango leaves has been described in the present study. The final products were also analyzed for their sensorial evaluations and antioxidative capacities. Sensory evaluation was done by a panel of 25 semi-trained tasters, using 9-point hedonic scale. Antioxidant profiling was done using common *in vitro* tests like DPPH and ABTS radical scavenging assays, contents of total polyphenolics and flavonoids, ferric reducing potentials and abilities for crocin bleaching of control and products. The most profound effect was observed in the polyphenolic contents of the samples as it was increased 7 to 10-times in the fortified products. ABTS and DPPH radical scavenging abilities were at par indicating balanced presence of highly or less polar bioactives. The radical scavenging aptitudes might be due to presence of flavonoids, which increased 2 to 3-times in the fortified products. There was a profound increment in the crocin bleaching abilities of the finished products. This indicated presence of both electron-donating and hydrogen atom donating biomolecules in the fortified products. No adverse indications were reported in their sensorial qualities.

KEYWORDS: Antioxidant, Mango, Jelly, Polyphenols, ABTS, DPPH.

INTRODUCTION

Jelly production is a good example of preservation and later consumption, with long storage periods that added the value of a fruit. Jelly production also allows the utilization of

underused fruits, such as secondary quality and over-ripe grapes that are usually not liked by consumers and, hence, are generally wasted.[1] A perfect Jelly should be transparent, well set, not too stiff and should have the flavour of the fruit. It should be of attractive colour and maintain its shape when taken out from the mould. It should be firm enough to retain a sharp edge but tender enough to quiver when pressed. It should not be gummy, sticky, or syrupy or have crystallised sugar. The product should be free from dullness with little or no syneresis and neither tough nor rubbery.[2]

Colors play an important role in enhancing the acceptability of food products. However, many food products suffered loss in color and texture due to food processing procedures such as heat treatment, pH changes, light exposure and storage condition. Therefore, synthetic food colorants are added to recover the loss and to enhance the appearance of food products. Unfortunately, some synthetic colorants has been report to be health hazardous.[3] As a result, natural pigment from biological sources came into consideration - especially plant pigments, which include betalain, anthocyanins, and other flavonoids, carotenoids and chlorophylls.[4]

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the World. Mango leaves, bark, and fruit (pulp, peel, and stone) are rich sources of bio-active compounds such as proteins, vitamin A, vitamin C, carotenoids, mangiferin, phenolic compounds, dietary fibre (DF), carbohydrates, minerals, and other anti-oxidants known to have medicinal, nutritional, and industrial benefits.[5] Mango pulp contains gallic acid and six hydrolysable tannins that constituted approximately 98% of the total polyphenols.[6] Also, the gallic acid content of mango pulp is one of the highest among tropical fruits. [7] That is why, a body of literature reported preparation of value added food products with mango components and bioactives.[8-10]

As discussed earlier, color and flavor loss in food products are one important setbacks to the consumers searching for better quality products. Earlier studies from our laboratory indicated that supplementation of natural coloring agents improve not only the texture and appearence of the food products, but also improved beneficial characteristics of the substances.[11] The present study was thus designed to use extracts of mango leaves as a source of natural color and important bioactives to be fortified in synthetically prepared jellies. This would provide an way to make the most of otherwise underutilized mango leaves. Both young and older leaves were used in the study to compare difference, if any, in their antioxidant properties. The goal was designed to be achieved by some common *in vitro* antioxidant tests, not only to

adjudicate quality of the products, but also to delineate mechanism of antioxidant actions of the finished products.

MATERIALS AND METHODS

Chemicals

2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), ABTS, were obtained from Sigma, USA. 2,2′-Diphenyl-1-picryl hydrazyl (DPPH) were obtained from Himedia, India. Folin-Ciocalteau reagent and gallic acid were obtained from Merck, India. 2,2′-azobis(2-Methylpropionamidine) dihydrochloride (AAPH), 2,4,6-Tris(2-pyridyl)-*s*-triazine (TPTZ) and Quercetin was procured from SRL, India. All other chemicals used were of AR grade. Deionized distilled water was used in the entire study.

Collection of samples

Fresh and older mango (*Mangifera indica*) leaves were obtained from household gardens of Barasat in 24 Parganas (N) district of West Bengal. These were washed with distilled water, followed by rinsing with deionized water. The samples were checked for dirt or any visible damages, and were discarded if found.

Preparation of jelly

Leaves were cut into small pieces before extraction. About 5 gms of leaf pieces were put in a domestic mixer-grinder with little water to extract the juice. The extractive was then filtered using a clean muslin cloth to get the juice. 20 ml of such juice was added to same volume of water and poured into a vessel to boil using a domestic LPG gas-burner. During boiling, gradually 50 gms of sugar, 0.2 gm of citric acid and 0.4 gm of agar were added with 2 minutes intervals. After boiling for another 10 minutes, the mixture was cooled, 1 ml of mango essence was added, filtered and kept at refrigerator overnight. The jelly was stored in dark in glass bottles for future evaluations.

ABTS radical decolorization assay

The ABTS assay was performed using a previously described procedure.[12] The oxidant, $ABTS^{-\bullet}$, was generated by per sulfate oxidation of 2,2′-azinobis(3-ethylbenzothiazoline)-6-sulfonic acid. This solution was diluted with phosphate buffer (pH 7.4) until the absorbance reached 0.7 to 0.8 at 734 nm in a Systronics spectrophotometer (model – 2202). The oxidant solution was mixed with the sample extracts in such a way that total volume of the solution reached 1 ml. The absorbance was read at room temperature, 4 minutes after mixing. Gallic

acid was used as positive control and the results were expressed as Gallic acid equivalents (µg/gm sample).

DPPH radical decolorization assay

The DPPH assay was performed using a previously described procedure.[13] 1 ml DPPH solution (3 mg DPPH powder in 25 ml ethanol) was mixed with 0.5 ml sample solution and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). Gallic acid was used as positive control and the results were expressed as µg of gallic acid per gram sample.

Total polyphenolics content assay

The assay was performed using a previously described procedure.[14] Briefly, 0.5 ml of sample was mixed with 1.5 ml Folin-Ciocalteau's solution (1:10 v/v diluted with distilled water) and allowed to stand for 28±2°C for 5 min. Then 2 ml of 7% (w/v) aqueous sodium carbonate solution was added and the mixture were allowed stand for another 90 min and at darkness. The absorbance of the blue color that developed was measured at 725 nm using spectrophotometer (Systronics, Model – 2202). Gallic acid was used to prepare the standard curve (20–100 µg/ml) and the total phenolic concentration in the spice extract was expressed as mg of gallic acid per gram sample.

FRAP assay

Ferric reducing antioxidant potential (FRAP) of the samples were estimated with a previously established procedure with minor modifications.[14] Briefly, a maximum of 100 µl of 60% ethanolic extracts of the sandesh or standard was mixed with 1.9 mL of FRAP reagent and incubated at 37^{o}C for 30 mins. FRAP reagent was prepared by mixing 50 mL of 0.1 M acetate buffer (pH 3.6), 5 mL of 10 mM TPTZ solution and 5 mL of 20 mM $FeCl_3$ solution. After the stipulated time period, absorbance was measured at 593 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid is used as standard. Results are expressed as Gallic acid equivalents (GAE, µg/gm sample).

Crocin bleaching assay

Crocin bleaching assay is based as result of oxidation of crocin induced by peroxyl radicals produced from thermal decomposition of azo-initiator, AAPH [2, 2'-azobis (2-amidinopropane) dihydrochloride] with minor modifications.[15] Crocin was extracted from dried stigmas of saffron. The test samples and standard were added to each test tube

individually containing 4 ml of ethanol, 75 µl 0.5M AAPH solution and 425 µl crocin extract to a total volume of 5 ml. These tubes kept incubated at room temperature for 60 min. After incubation, absorbance of these reaction mixture tubes was measured at 443 nm. Control was prepared by mixing the reagents without test samples or standard and absorbance was determined immediately. The percentage of crocin bleached by extract was calculated using the following formula: Bleached crocin % = {[(A_c─A_e)/A_c]x 100}, where, A_c = absorbance of control and A_e = absorbance of sample.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging potentials of the samples were estimated with a previously described procedure.[16] Briefly, 10 mM each of $FeSO_4.7H_2O$, EDTA, 2-deoxy-D-ribose and H_2O_2 solutions were prepared in water. Each solution of above four with sample/standard solution was mixed in a test tube to get a final volume of 1 ml and incubated at 37^oC for 90 mins. H_2O_2 solution was added last. After the incubation, 2.8% (w/v) aqueous TCA solution and 1% (w/v) aqueous TBA solution were added to the reaction mixture and kept at boiling water bath for 20 mins. Development of the pink chromophore was measured at 532 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Results were expressed as Gallic acid equivalents (GAE).

Estimation of total flavonoids

Colorimetric aluminum chloride method was used for flavonoid determination following a published procedure.[17] Briefly, 0.5 ml extractive of each sample were mixed with 1.5 ml of methanol, 0.1 ml of 10% (w/v) aluminum chloride, 0.1ml of 1 M potassium acetate solution, and 2.8 ml of distilled water, and left at room temperature for 30 minutes. The absorbance of the reaction mixture was measured at 415 nm with a double beam UV-Vis spectrophotometer (model – Systronics 2202). Total flavonoids content were calculated using a calibration curve of quercetin as standard and the results were expressed as µg quercetin equivalent/gm sample.

Sensory evaluation

Twenty five panelists were selected from staff, faculty and students of the Department to evaluate the acceptability of the products, on the basis of color flavor and taste, in comparison to the control. They evaluated the samples on the basis of 9-point hedonic scale, ranging from ‘like extremely = 9’ through ‘like or dislike = 5’ to ‘dislike extremely = 1’.[11]

The panelists were satisfactorily trained to circumvent any biasing during the assessment of the sample. Each panelist assessed all the test samples as well as the controls. The entire experiment was repeated four times.

Statistical Analyses

All the experiments were performed in quadruplicate and data were presented as mean ± standard deviation. The analyses were done with the software 'SPSS Statistics 17.0' (SPSS Inc. USA).

RESULTS AND DISCUSSION

Results of ABTS assay indicated that mango leaves supplemented jellies were substantially better radical scavengers than the control jelly, which was devoid of mango leaves extract (Fig. 1). Between the new mango leaves jelly and old mango leaves jelly, the later supplementation proved to be better in respect of radical scavenging, although the differences were marginal.

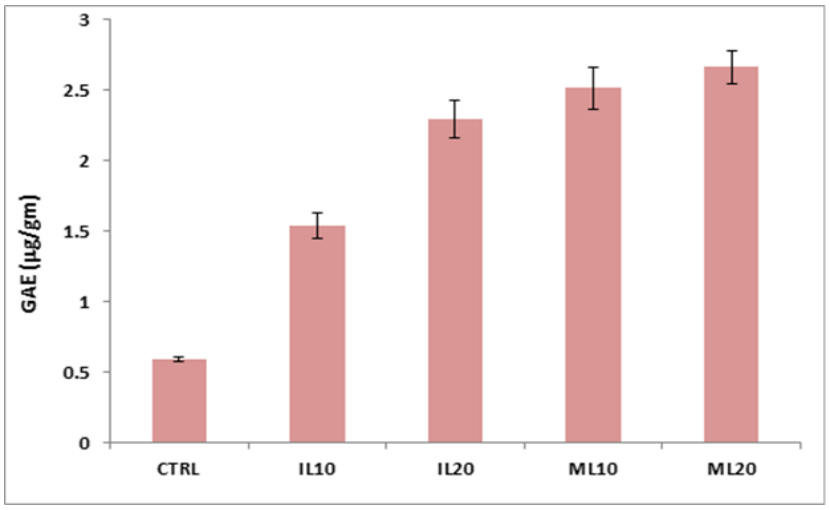

Fig 1: ABTS radical scavenging potential of the jellies supplemented with mango leaf extracts. Data are expressed as Mean ± SD (*n*=4). Results are expressed as gallic acid equivalents (GAE). CTRL – control, IL10 & IL20–immature leaf extract supplementation 10% & 20% w/w, respectively, ML10 & ML20 – mature leaf extract supplementation 10% & 20% w/w, respectively.

Similar results were obtained with the DPPH assay. Mango leaves supplemented jellies were better radical scavengers than the control jelly (Fig. 2). Between the new mango leaves jelly and old mango leaves jelly, the later supplementation proved to be better in respect of radical scavenging, although the differences were marginal.

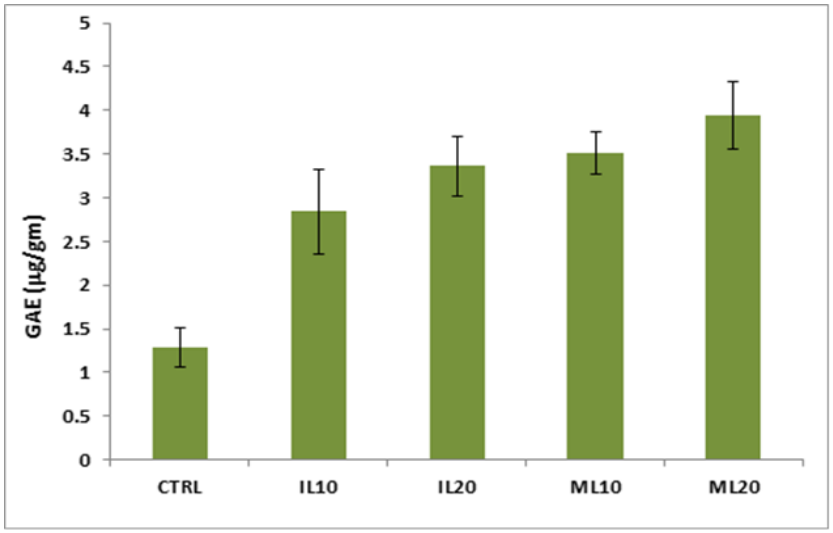

Fig 2: DPPH radical scavenging potential of the jellies supplemented with mango leaf extracts. Data are expressed as Mean ± SD (*n*=4). Results are expressed as gallic acid equivalents (GAE). CTRL – control, IL10 & IL20–immature leaf extract supplementation 10% & 20% w/w, respectively, ML10 & ML20 – mature leaf extract supplementation 10% & 20% w/w, respectively.

The above two assays indicate presence of highly polar and less polar antioxidant molecules in the test substances. Scrutinizing the present results, it could be concluded that the jellies were replete with both types of bioactives, which could be beneficial to the humans after consumption. Fairly well DPPH radical scavenging abilities were reported earlier in processed sapota pulp jelly, where it was observed that radical scavenging abilities were reduced with respect to sapota pulp extracts.[18] Similarly, supplementation with pomegranate peel improved antioxidant property of jelly although it did not have any effect on stability over time.[19]

Phenolics have been reported to exhibit antioxidant activity, due to their phenol moiety, and have the ability to scavenge free radicals, probably via hydrogen atom or electron donation. Phenolics contents were furnished in Fig. 3. The results indicated betterment in phenolic contents in the supplemented jellies. Moreover, phenolic contents were substantially better if the jellies could be supplemented with extracts of immature leaves. Supplementation of phenolics have previously proved to be effective against alleviating chronic liver diseases as tested in animal models.[20] The present product might have some positive effect if utilized for human consumption.

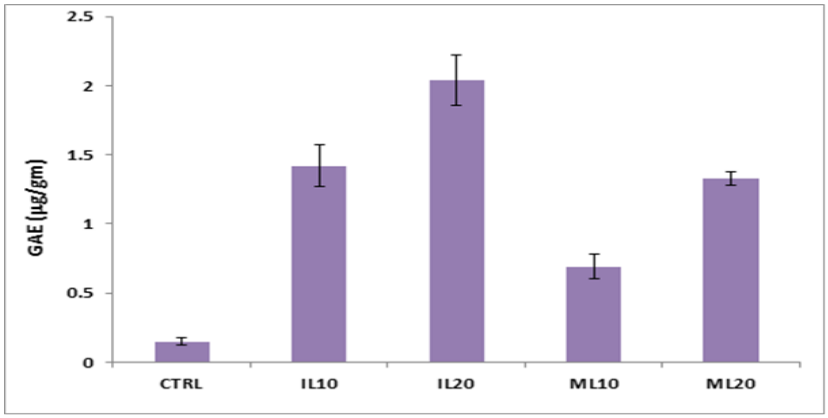

Fig 3. Total phenolic contents of the jellies supplemented with mango leaf extracts. Data are expressed as Mean ± SD (*n*=4). Results are expressed as gallic acid equivalents (GAE). CTRL – control, IL10 & IL20–immature leaf extract supplementation 10% & 20% w/w, respectively, ML10 & ML20 – mature leaf extract supplementation 10% & 20% w/w, respectively.

The FRAP assay is typically used to determine antioxidant activity as the method is simple and quick. Besides that, the reaction is reproducible and linearly related to molar concentration of the antioxidants. Higher FRAP values give higher antioxidant capacity because FRAP value is based on reduction of ferric ion, where antioxidants are the electron donating reducing agents. Results of this assay suggested that the test substances were substantially supplemented with electron donating antioxidants present in mango leaves (Fig. 4). Supplementation was better when immature leaves were used for extract preparation.

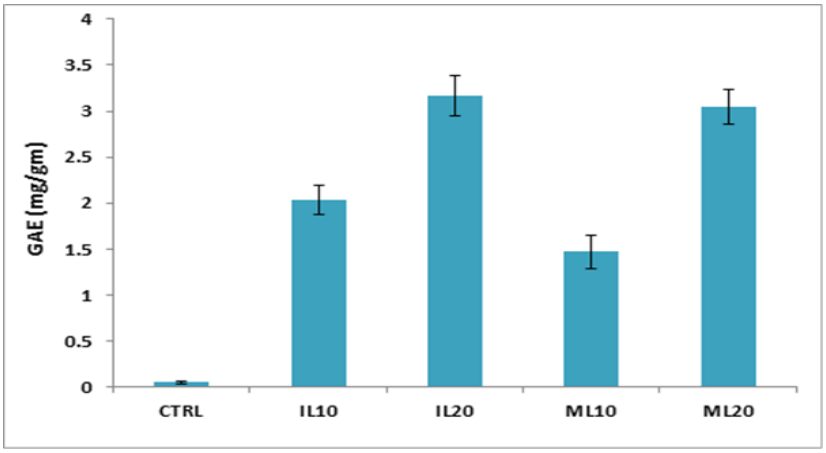

Fig 4. FRAP assay of the jellies supplemented with mango leaf extracts. Data are expressed as Mean ± SD (*n*=4). Results are expressed as gallic acid equivalents (GAE). CTRL – control, IL10 & IL20–immature leaf extract supplementation 10% & 20% w/w, respectively, ML10 & ML20 – mature leaf extract supplementation 10% & 20% w/w, respectively.

Crocin bleaching assay was performed to delineate presence of hydrogen atom donating antioxidants in the mango leaves extracts supplemented jellies. From this assay, it can be seen that supplementation of immature and mature mango leaves extracts at 10% and at 20% concentrations showed better antioxidant properties than the control jelly (Fig. 5). Crocin bleaching was dose-dependent. Usually phenolics donate hydrogen atoms from the free hydroxyl radicals present in the molecules. It could be implicit that the samples were supplemented with hydrogen donating antioxidants, along with electron donating antioxidants that was obviated by FRAP assay.

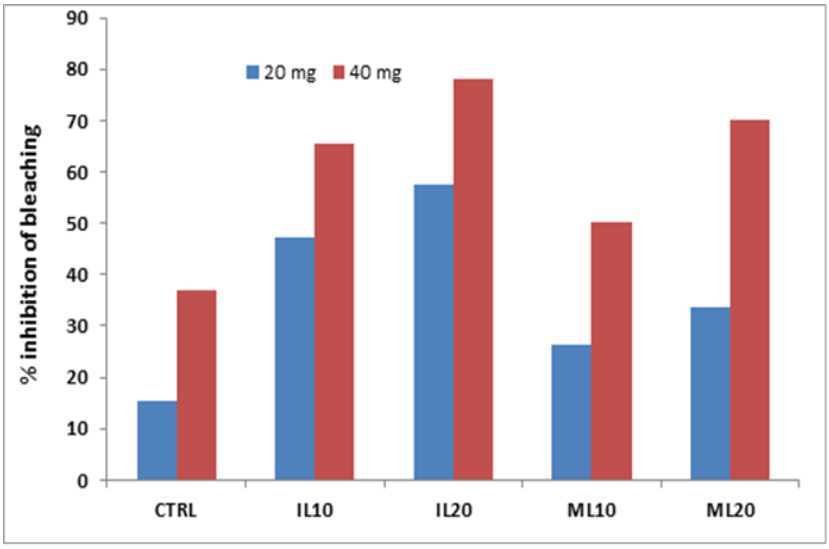

Fig 5. Crocin bleaching protection capacities of the jellies supplemented with mango leaf extracts. CTRL – control, IL10 & IL20–immature leaf extract supplementation 10% & 20% w/w, respectively, ML10 & ML20 – mature leaf extract supplementation 10% & 20% w/w, respectively.

The results of the hydroxyl radical scavenging assay commensurated with crocin bleaching abilities of the samples. It was observed that supplemented jellies were better scavengers of this deleterious radical than control (Fig. 6). Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous biological damage. Since hydroxyl radicals were efficiently neutralized, we may conclude that the hydrogen atom donors, presence of which was indicated in Crocin bleaching assay, play an important role in such activities.

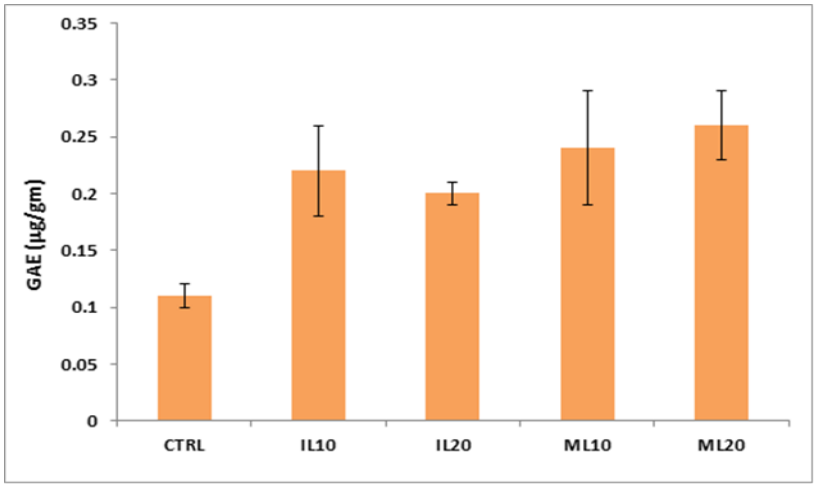

Fig 6. Hydroxyl radical scavenging abilities of the jellies supplemented with mango leaf extracts. Data are expressed as Mean ± SD (*n*=4). Results are expressed as gallic acid equivalents (GAE). CTRL – control, IL10 & IL20–immature leaf extract supplementation 10% & 20% w/w, respectively, ML10 & ML20 – mature leaf extract supplementation 10% & 20% w/w, respectively.

As flavonoids are the principle bioactives present in leaves, contents of the bioactive were measured in the test jellies. Results are furnished in Fig. 7. Flavonoid contents were greater in test jellies with respect to control, and also the contents were dose dependent, clearly indicating that these bioactives played an important role in the antioxidative abilities of the jellies.

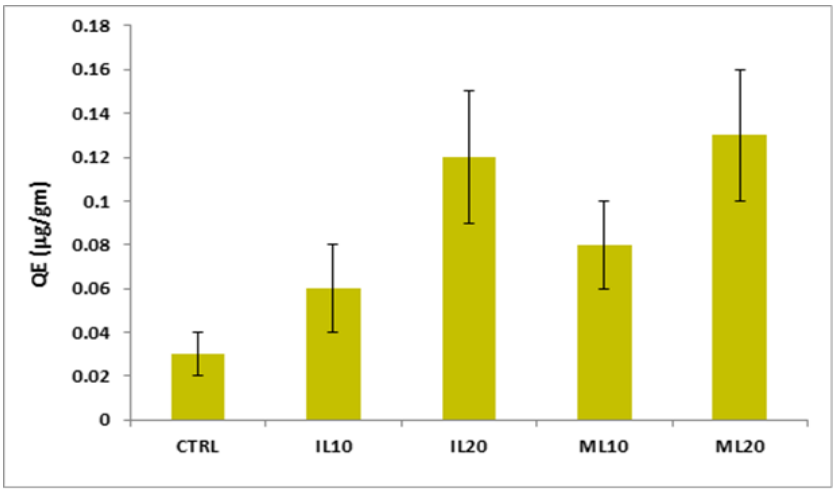

Fig 7. Flavonoids contents of the jellies supplemented with mango leaf extracts. Data are expressed as Mean ± SD (*n*=4). Results are expressed as quercetin equivalents (QE). CTRL – control, IL10 & IL20–immature leaf extract supplementation 10% & 20% w/w, respectively, ML10 & ML20 – mature leaf extract supplementation 10% & 20% w/w, respectively.

Sensory evaluation of the supplemented jellies revealed that the product has slight declining preference towards the tasters, although the results in the 9-point scale was well within the deviation limit (Fig. 8). The tasters reported slight bitterness in the test substances although it did not affect the overall choice of the products. There was no significant difference detected between the appearance, taste and sweetness of the test vis-a-vis control jellies. It was also observed that there was no difference between uses of immature or mature leaves on the preparation of the jellies.

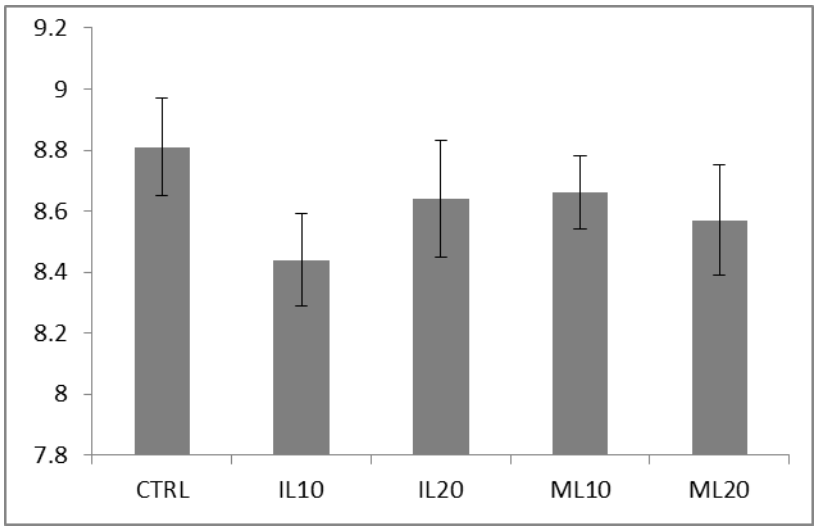

Fig 8. Sensory evaluation of the jellies supplemented with mango leaf extracts. Data are expressed as Mean ± SD (*n*=4). CTRL – control, IL10 & IL20–immature leaf extract supplementation 10% & 20% w/w, respectively, ML10 & ML20 – mature leaf extract supplementation 10% & 20% w/w, respectively.

Mango leaves are a rich source of phenolic compounds and popular for their antioxidant capacity. Fortification of leaf extracts thus tends to improve antioxidant quality of the jellies prepared with them. However, Storage of fruit extract supplemented jams showed tendency of reduction of the content of phenolic compounds compared to their content of products analysed directly after production.[21] Nevertheless, a substantial amount of antioxidants would still remain in the finished products to exert their physiological activities. The present study is a step towards such endeavor.

CONCLUSION

The present study concluded that jellies fortified with mango leaf extracts have potential antioxidant activities. Such jellies had been shown to be supplemented with phenolic compounds, especially flavonoids, which have reducing activities and the abilities to

scavenge free radicals. These supplemented jellies had greater hydroxyl radical scavenging activities implicating their potential beneficial role in human physiological systems. Flavonoid contents in the jellies containing mature or immature leaf extracts remained same, indicating the fact that both types of leaves could be used to prepare the value added product. Sensory evaluation study indicated that panelists liked the control jelly slightly more than the jellies prepared with mango leaf extracts, although the results in the 9-point scale was well within the deviation limit. It might be due to the astringency present in almost all leaves. However, mango leaves are cheap and easily available clearly indicating the fact that preparation of fortified jellies would be economical. The current study indicated that consuming jellies fortified with mango leave extracts as a part of healthy dessert would promote greater well-being rather than eating plain jellies.

ACKNOWLEDGEMENT

The authors are grateful to Sarada Ma Girls' College authority (under Ramakrishna Vivekananda Mission) for providing financial and infrastructural assistance.

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