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Quantification of phenolic compounds and antioxidant capacity of an underutilized Indian fruit: Rayan [*Manilkara hexandra* (Roxb.) Dubard]

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Abstract

The fruit of *Manilkara hexandra* (Roxb.) Dubard is one of the most underutilized fruits of India and in Gujarat state. It is popularly known as 'Rayan'. The fruit and seed of Rayan were analysed for their total phenolic and flavonoid content, phenolic compounds and total antioxidant capacity with six different assay methods. The results indicated that the methanolic extract of Rayan fruit being a good source of phenolic (811.3 mg GAE/100 g fw) and flavonoid (485.56 mg RE/100 g fw) content. Also, eleven known phenolic compounds were tentatively identified for the first time from the fruit and seed of Rayan. The LC–MS/MS analysis of fruit revealed the presence of major phenolic compounds such as gallic acid, quercetin and kaempferol, while quercetin, gallic acid and vanillic acid in seed. The presence of quercetin suggests health benefits. The fruit of Rayan was also proved to be a better source of antioxidants as measured by FRAP, RPA, DPPHRSA, ABTSRSA and HRSA except NORSA in comparison with that of seed. The current study explains that *M. hexandra* is a relatively good source of antioxidants such as phenols and flavonoids for diet.

Keywords: *Manilkara hexandra* (Roxb.) Dubard; Underutilized fruit; High performance liquid chromatography–mass spectrometry; Phenolic compounds; Total antioxidant capacity

1. Introduction

Evidence is increasing regarding a range of a diet-chronic disease link. Thus, nutrition research has shifted from focusing exclusively on alleviating nutrient deficiencies to prevent or reduce morbidity and mortality due to chronic diseases [1,2]. Along with the daily diet, increased fruit and vegetable consumption has been promoted and correlated immensely not only for their nutritional content but also for their potential health functionality against various degenerative diseases such as cancer, cardiovascular, cataract, diabetes, and neurodegenerative diseases like Alzheimer's and Parkinson's [3]. This phenomenon has been attributed to the protective effects of antioxidant components contained in fruit, which are a rich source of natural enzymatic and non-enzymatic antioxidants [4]. In addition to the traditional nutrient antioxidants (*e.g.*, vitamins C and E and β-carotene), fruits contain polyphenolic (*e.g.* flavonoids)

Abbreviations: ABTS, 2,2 azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt; ABTSRSA, ABTS radical scavenging activity; DPPH, 1,1-diphenyl-2-picryl-hydrazyl; DPPHRSA, DPPH radical scavenging activity; dw, dry weight; FRAP, ferric reducing antioxidant power assay; fw, fresh weight; HRSA, hydroxyl radical scavenging activity; NORSA, nitric oxide radical scavenging activity; RPA, reducing power assay; TE, Trolox equivalent; TPTZ, 2,4,6-tris (2-pyridyl)-s-triazine; Trolox, 6-hydroxy-2,5,7,8-tetra methylchromane-2-carboxylic acid.

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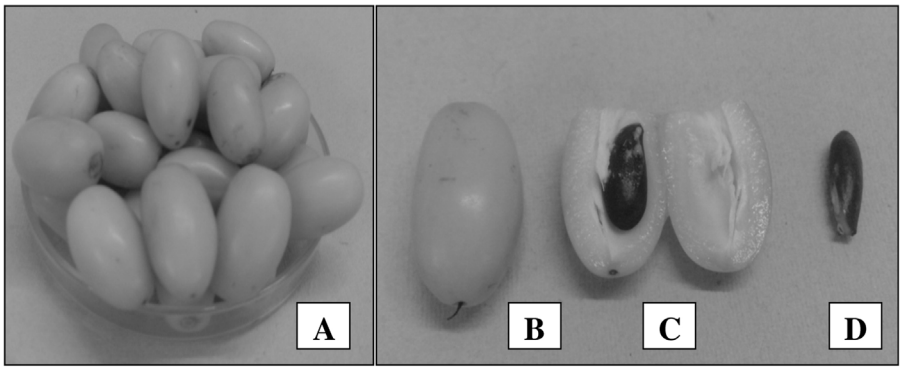

Fig. 1. Whole fruits of Rayan [*Manilkara hexandra* (Roxb.) Dubard] (A), single fruit (B), section (C) and seed (D).

compounds, which may play an important role in the overall antioxidant activity of fruits [5].

Manilkara hexandra (Roxb.) Dubard [Synonym: *Mimusops hexandra* (Roxb.)] belongs to Sapotaceae family. It is a socio-economically important underutilized fruit species characterized by the presence of sticky, usually white latex in the cuts of bark, branches, leaves and fruit. The small to medium sized evergreen trees are mostly found in western and central Indian states of Rajasthan, Gujarat, Madhya Pradesh and Maharastra [6]. The matured fresh fruits are obovoid-oblong to ellipsoid in shape, measuring about 1–1.5 cm wide (Fig. 1), one or two seeded, shining yellow colored which is soft and sweet in taste, being a good source of minerals and vitamins with low fat content [7,8]. It is locally known as 'Rayan' or 'Khirni'. The fruit and other parts of the tree (bark, stem bark, root, leaves and latex) are known for their various nutritional and medicinal properties used by the older and tribal peoples of India. It has been shown that, this fruit is nutritionally necessary for a well-balanced diet as the methanolic extract of fruits are reported to have significant hypoglycemic effect and can be use in the management or control of type II diabetes [9]. In addition, the acetone fraction of *M. hexandra* seed is reported to contain the crude saponin mixture with significant anti-inflammatory activity [10]. The seeds of *M. hexandra* contain approximately 25% edible oil, which can be used for cooking purposes [11]. It is known to have high remedial value as it is demulcent and emollient [12]. Parikh et al. [13] studied the nutritional profile of an underutilized Indian fruit of Rayan.

Despite very few literatures showed the importance and medicinal use of various parts of Rayan tree, it still represents the lack of data regarding identification of phenolic compounds and antioxidant capacity. In this context, the present study was planned for the identification of phenolic compounds, total phenolic content, flavonoid content and total antioxidant capacity using various assay methods from fruit and seed of Rayan.

2. Materials and methods

2.1. Chemicals

ABTS (A 1888), L-ascorbic acid (95210), DPPH (D 9132), gallic acid (G 7384), *N*-(1-naphthyl ethylenediamine dihydrochloride) (N 9125), 1,10-phenanthroline (320056), rutin hydrate (R 5143), sulphanilamide (33626), TPTZ (T 1253) were of Sigma–Aldrich brand, while sodium nitroprusside (dihydrate) (71778) and Trolox (56510) were of Fluka brand (Sigma–Aldrich, Bangalore, India). Other chemicals used were of analytical grade. The solvents used for chromatography were of HPLC grade.

2.2. Sample

2.2.1. Sample preparation

The fruits of Rayan (*M. hexandra*) (one kilogram of fresh, ripe but firm fruits, free from bruises) were purchased from the local market of Anand (Gujarat, India). They were thoroughly washed under running tap water, cut in halves, deseeded to get the edible part and crushed in a mixer. Its freshly collected seeds were also washed, oven dried and powdered using a kitchen grinder.

2.2.2. Sample extraction for phenolic content and antioxidant determination

The fruit pulp and seed powder of Rayan were used for the extraction for the determination of phenolic content and antioxidant. The 5.0 g and 2.0 g of fruit and seed samples respectively were extracted thrice in 80% aqueous methanol (pH 2.0) by shaking at room temperature for 90 min. Supernatants were centrifuged, filtered and the volume of each of these samples was made up to 50 ml with the solvent using a volumetric flask. The extracts were stored at −20 °C for the estimation of total phenolic content and total antioxidant capacity. The fruit and seed samples were extracted in duplicate batches with two separate purchases in the same season and four observations of two different experiments were analyzed statistically.

2.3. Determination of total phenol

2.3.1. Total phenolic content

Total phenolic content was determined using Folin–Ciocalteu method [14]. Gallic acid was used as a standard and the results are expressed in milligrams of gallic acid equivalent per 100 g sample (mg GAE/100 g).

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