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HPTLC STUDIES OF PHENOLIC ACID CONTENT IN UNRIPE AND RIPE VARIETIES OF *MANGIFERA INDICA* L. (ANACARDIACEAE)

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ABSTRACT: Mango is the most popular fruit having excellent flavor, pleasant aroma, attractive color and taste. It is a good source of vitamin A and C, TSS (total soluble solids) and minerals. It is also a medium source of carbohydrate. Their fruit has distinct physiomorphological characteristic features and composition variation. Phenolics are aromatic benzene ring compounds with one or more hydroxyl groups produced by plants mainly for protection against stress. Phenolics play important roles in plant development, particularly in lignin and pigment biosynthesis. Phenolic are among the major contributors that are accountable for antioxidant properties in fruits, vegetables, whole grains and other plant-based materials. Hence in the present study, phenolic acid analysis was done by HPTLC for comparison between three varieties at unripe and ripe stages. Three varieties namely alphonso, kesar and rajapuri were studied extensively for the phenolic acid variations. Phenolic acids occur naturally in plants and are their main polyphenols. In plants, they act as signaling molecules and agents of defense. In humans, these compounds act as antioxidants which prevent free radical damage in the body and also prevent diseases such as cancer. Four phenolic compounds were detected after the analysis.

INTRODUCTION: In addition to many essential nutritional components, plants contain phenolic substances, a large and heterogeneous group of biologically active non-nutrients [1]. Phenolic acids present in plants are hydroxylated derivatives of benzoic and cinnamic acids [2]. Phenolic compounds are also important in the defence mechanisms of plants under different environmental stress conditions such as wounding, infection, and excessive light or UV irradiation [3, 4].

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Phenolic compounds, ubiquitous in plants, are an essential part of the human diet and are of considerable interest due to their antioxidant properties and potential beneficial health effects. These compounds range structurally from a simple phenolic molecule to complex high-molecular-weight polymers.

The biological potency of secondary plant phenolics was found empirically already by our ancestors; phenolics are not only unsavoury or poisonous, but also of possible pharmacological value [5]. Moreover, the bioavailability of flavonoids and phenolic acids from various foods, and the extent and mechanism of absorption in the human body are poorly known. Mangoes *(Mangifera indica* L.) are rich sources of dietary fiber, vitamin

C, and phenolic antioxidants [6, 7, 8, 9] and numerous studies have been conducted on the potential nutritional and health-effects of this fruit [10 - 17]. Of particular interest are the high concentrations of many diverse phenolic compounds in mango, especially in the peel and kernels. The antioxidant contents of tropical fruits such as mangoes are taking greater importance in evaluations of fruit quality and in marketing. Hence the study was designed to evaluate different phenolic acids present in mango varieities at unripe and ripe stage. In this study, three varieties Alphonso (3AN- stage 3 Alphonso navsari, 4AN- stage 4 Alphonso navsari), Kesar (3KN- stage 3 Kesar Navsari, 4KJ- stage 4 Kesar Junagadh) and Rajapuri (4RJ- stage 4 Rajapuri Junagadh, 4RN- stage 4 Rajapuri Navsari) at two stages (stage 3 (unripe fruit) and 4(ripe fruit)) of *M. indica* were considered for the study.

MATERIALS AND METHODS: Fruit of mango cultivars were obtained from orchard of Junagadh and Navsari Agriculture University (Gujarat, India). Sampling from 20 trees within an orchard was carried out. Flesh firmness was determined by removing peel on one shoulder (about 3 cm^2) of each of 10 fruits. Accurately weighed 5 g of fresh fruit pulp of two varieties of *Mangifera indica* L. were extracted with ethanol and the extracts were concentrated to a final volume of 15 ml. Thin layer chromatography (TLC) is an important analytical tool in separation, identification and estimation of different classes of natural products. The variation in phenolic content of fruit pulp of three different varieties of *M. indica* was determined by TLC study. All solvents and chemicals used were of analytical grade. silica gel $60F_{254}$ TLC plates were purchased from Merck (Darmstadt, Germany).

Chromatographic Conditions: A Camag TLC system equipped with Camag Linomat V an automatic TLC sample spotter was used for the analysis. Chromatography was performed using pre-activated (60 ºC for 5 min) silica gel $60F_{254}$ TLC plates (10 × 10 cm; layer thickness 250 µm). Samples were applied on the plate as 8 mm wide bands with an automatic TLC sampler under a flow of N_2 gas, 10 mm from the bottom and 10 mm from the side and the space between two spots were 15 mm of the plate. The linear ascending development was carried out in a Camag twin trough chamber (10 ×10 cm) saturated with 20 ml mobile phase of acetone: chloroform: *n*-butanol: glacial acetic acid: water (60:40:40:40:35, v/v/v/v/v) for 20 min at room temperature (25 ± 2 ºC and 40% relative humidity). The plates were developed up to 8 cm under chamber saturation conditions. Subsequent to the development, TLC plates were dried in current air with the help of a hair dryer. The post chromatographic derivatization was carried out with fast blue salt reagent followed by heating at 110 ºC for 10 min. Evaluation of the plates were performed with Camag scanner 3 (win CATS 4.0 integration software). Densitometric scanning was performed in the absorption-reflection mode at 540 nm, using a slit width of 6 × 0.45 mm and data resolution 100 µm step and scanning speed 20 mm/s with a computerized Camag TLC scanner.

Preparation of Fast Blue Salt Reagent: Fast blue salt (FBS) is 3, 3'-dimethoxy biphenyl 4,4'-bis (diazonium) -chloride. 0.5 g of FBS was dissolved in 100 ml of water.

Analysis of Samples: Fifteen microlitre of sample solutions were applied on a TLC plate developed and scanned as above. Peak areas of the detected phenolic compounds were recorded. TLC analysis of all samples was carried out thrice and their mean values of peak area were calculated. The mean peak area values were compared within the samples to determine the variations in phenolic content.

Specificity: The spot with same R_f value in each sample was confirmed as same compound by their spectral comparison. The peak purity was assessed by comparing the spectra at peak start, peak apex and peak end positions of the each spot.

RESULTS AND DISCUSSION: Mango has strong anti-oxidant activities, as it contains much amount of ascorbic acid, carotene, quercetin, phenols [18]. Phenolic compounds are not evenly distributed in fruits either at the sub cellular level or in the tissues. Accumulation of soluble phenolic compounds is greater in the external tissues of fleshy fruits (epidermal and sub epidermal layers) than in the internal tissue (mesocarp and pulp) [19].

Since the formation of phenolic compounds depends on light, they are mainly found in the skins of fruits. Accumulation of phenolic compounds varies strongly in relation to the physiological state

of the fruit, being a result of equilibrium between biosynthesis and further metabolism including turnover and catabolism. The most important control mechanisms in the phenolic metabolism include the amount of enzymes, regulation of enzyme activities, compartmentation of enzymes, availability of precursors and intermediates, and integration in the differentiation and development programs [20].

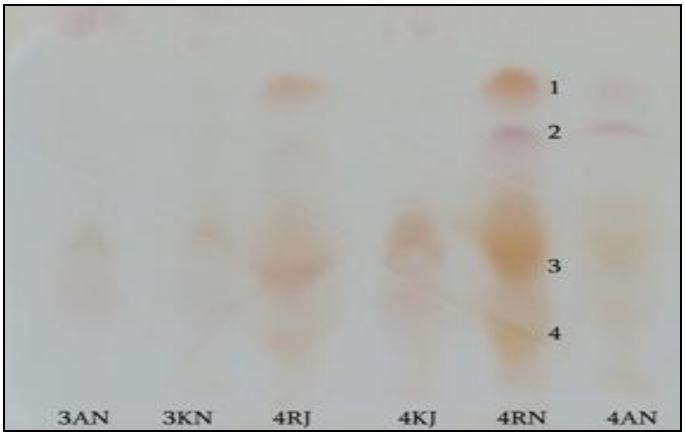

FIG. 1: PHOTOGRAPH OF THE TLC PLATE SHOWING 4 MAJOR PHENOLIC COMPOUNDS IN DIFFERENT VARIETIES OF *M. INDICA*

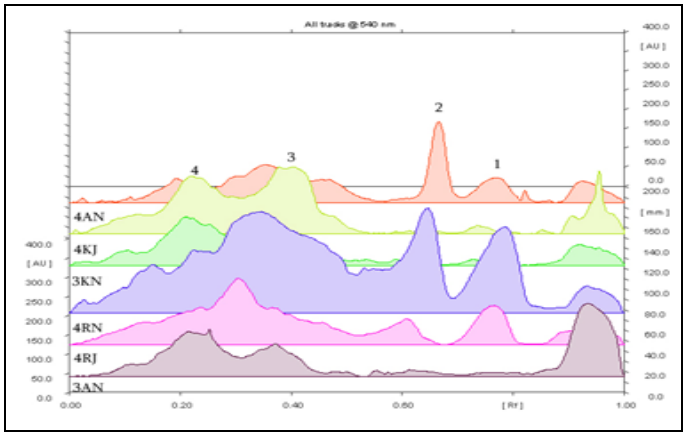

FIG. 2: DENSITOGRAM OF DIFFERENT VARIETIES OF *M. INDICA* SHOWING FOUR MAJOR PHENOLIC COMPOUNDS
1-compound I; 2-compound II; 3-compound III, and 4-compound IV.

Different compositions of mobile phase were tested and the desired separation of different phenolic compounds with a symmetrical and reproducible peak was achieved by using the mobile phase of acetone: chloroform: *n*-butanol: glacial acetic acid: water (60:40:40:40:35, v/v/v/v/v). After post chromatography derivatization with FBS, 4 major phenolic compounds were detected **Fig. 1** and **2**.

The details are as follows; Compound I (R_f 0.75 ± 0.02); compound II (R_f 0.64 ± 0.02); compound III (R_f 0.36±0.02) and compound IV (R_f 0.21 ± 0.02). Compound I was detected in 4RJ, 4RN, 3KN, 4KJ and 4AN variety of *M. indica* **Table 1**. 3AN, 4RJ, 4RN and 4AN varieties showed the presence of compound II **Table 2**. All the varieties studied showed the presence of compound III and IV **Table 3** and **4**. The compounds (I, II, III and IV) detected in different varieties were confirmed as same compounds by R_f value and spectral analysis **Fig. 4**, **6**, **8** and **10**.

TABLE 1: MEAN PEAK AREA FOR COMPOUND I IN DIFFERENT VARIETIES OF *M. INDICA*

Varieties of *M. indica*	R_f	Peak area
3AN	ND	ND
4RJ	0.76	6138.75±684.35***
4RN	0.77	11539.25±457.85***
3KN	0.74	695.6±224
4KJ	0.74	573.35±92.05
4AN	0.76	2349.35±328.25

Values are expressed as Mean ± SEM; n=3; ***-p<0.001; ND-not detected.

TABLE 2: MEAN PEAK AREA FOR COMPOUND II IN DIFFERENT VARIETIES OF *M. INDICA*

Varieties of *M. indica*	R_f	Peak area
3AN	0.65	247.45±67.67
4RJ	0.62	2481.1±290.9
4RN	0.65	11649.2±2063***
3KN	ND	ND
4KJ	ND	ND
4AN	0.66	5107.75±532.1

Values are expressed as Mean ± SEM; n=3; ***-p<0.001; ND-not detected.

Peak area is directly proportional to the quantity of the compound; hence the mean peak area of the compound was used to find the variation in the quantity of these detected phenolic constituents among different varieties of *M. indica*. Comparatively 4AN variety showed the least quantity of phenolic compounds. Significant higher quantity of phenolic compounds was found in 4RN. All the other varieties 3AN, 4RJ, 3KN and 4KJ showed moderate presence of these phenolic compounds **Fig. 3**, **5**, **7** and **9**. The variety 3AN showed absence of compound I.

Similarly, 3KN and 4KJ also showed the absence of compound II. The absence of compounds in these varieties do not assure the absolute absence, may be the compounds are below the limit of detection at this concentration. 4RN showed significant quantity of phenolic compound I and II. Compound III and IV predominated in 4KJ and

3AN varieties respectively. Of the studied varieties of *M. indica*, relatively 4RN showed higher phenolic content and 4AN showed the least quantity of phenolic constituents.

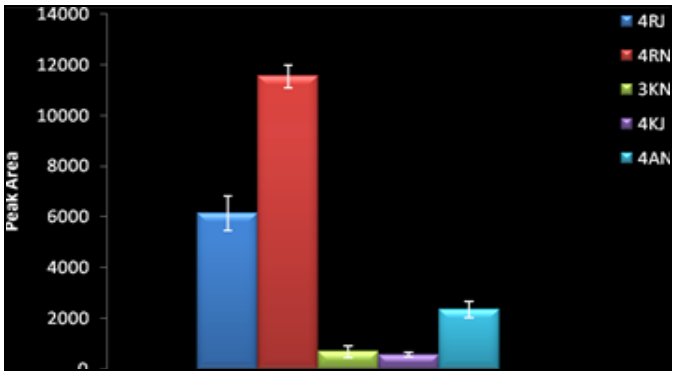

FIG. 3: COMPARISON OF MEAN PEAK AREA FOR COMPOUND I IN DIFFERENT VARIETIES OF *M. INDICA*

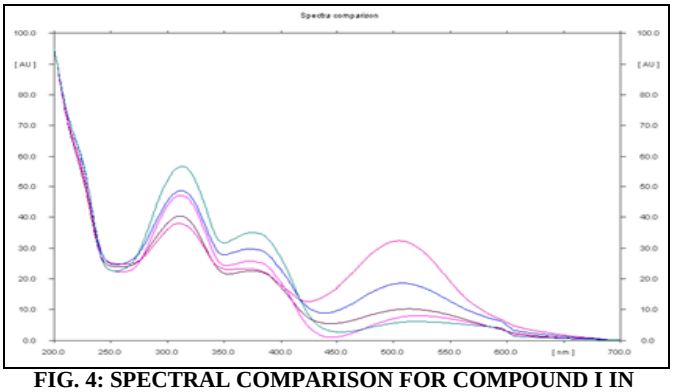

FIG. 4: SPECTRAL COMPARISON FOR COMPOUND I IN 4RJ, 4RN, 3KN, 4KJ AND 4AN VARIETIES OF *M. INDICA*

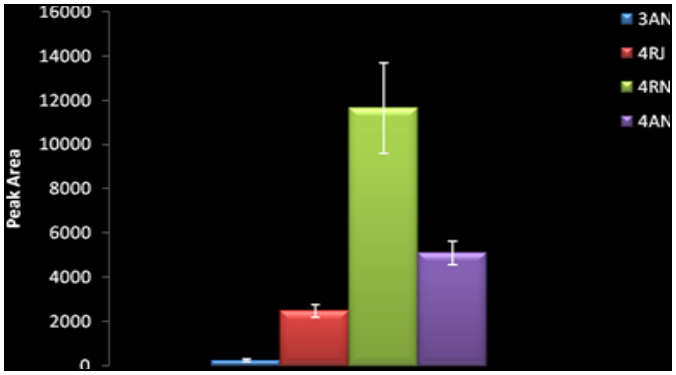

FIG. 5: COMPARISON OF MEAN PEAK AREA FOR COMPOUND II IN DIFFERENT VARIETIES OF *M. INDICA*

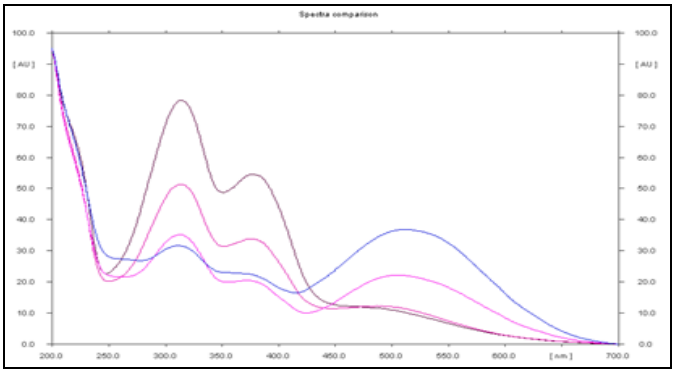

FIG. 6: SPECTRAL COMPARISON FOR COMPOUND II IN 3AN, 4RJ, 4RN AND 4AN VARIETIES OF *M. INDICA*

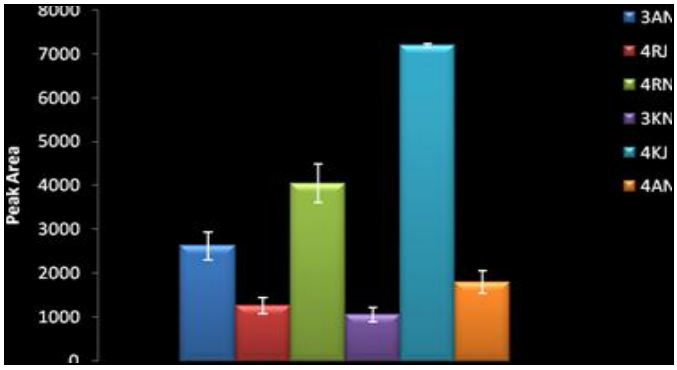

FIG. 7: COMPARISON OF MEAN PEAK AREA FOR COMPOUND III IN DIFFERENT VARIETIES OF *M. INDICA*

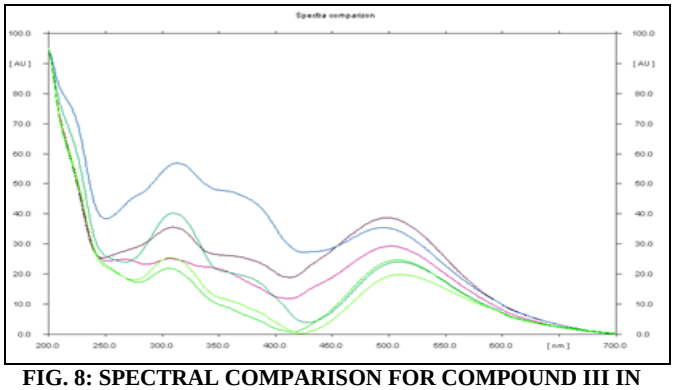

FIG. 8: SPECTRAL COMPARISON FOR COMPOUND III IN 3AN, 4RJ, 4RN, 3KN, 4KJ AND 4AN VARIETIES OF *M. INDICA*

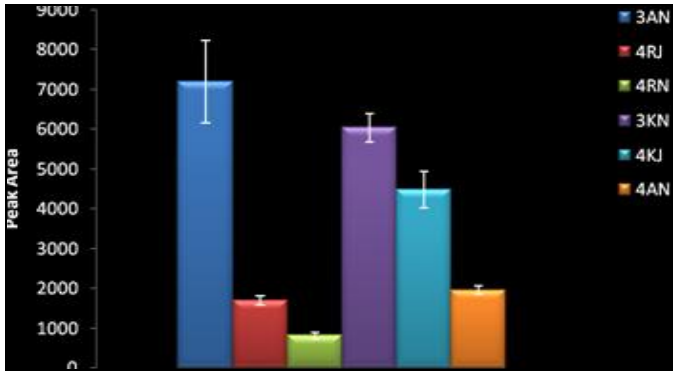

FIG. 9: COMPARISON OF MEAN PEAK AREA FOR COMPOUND IV IN DIFFERENT VARIETIES OF M. INDICA

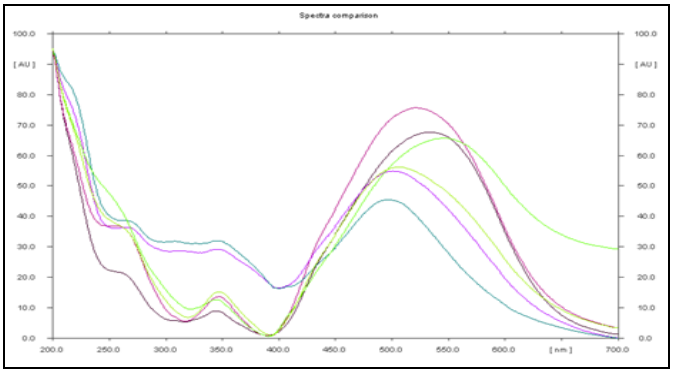

FIG. 10: SPECTRAL COMPARISON FOR COMPOUND IV IN 3AN, 4RJ, 4RN, 3KN, 4KJ AND 4AN VARIETIES OF *M. INDICA*

TABLE 3: MEAN PEAK AREA FOR COMPOUND III IN DIFFERENT VARIETIES OF *M. INDICA*

Varieties of *M. indica*	R_f	Peak area
3AN	0.37	2626.1±324
4RJ	0.37	1262.55±1262.55
4RN	0.35	4049.15±4049.15***
3KN	0.36	1059.5±163.6
4KJ	0.37	7195.95±37.55***
4AN	0.36	1798.95±257.25

Values are expressed as Mean ± SEM; n=3; ***-p<0.001; ND-not detected.

TABLE 4: MEAN PEAK AREA FOR COMPOUND IV IN DIFFERENT VARIETIES OF *M. INDICA*

Varieties of *M. indica*	R_f	Peak area
3AN	0.21	7195.6±1041.6***
4RJ	0.22	1700.75±113.55
4RN	0.22	815.3±85.7
3KN	0.23	6040.85±358.75***
4KJ	0.21	4485.35±463.15
4AN	0.20	1957.6±100.3

Values are expressed as Mean ± SEM; n=3; ***-p<0.001; ND-not detected

CONCLUSION: The present study gives an overview of various phenolic compounds present in unripe and ripe mango varieties. Phenolic compounds play a major role in plant defence and also scavange free radicals from living cells. HPTLC fingerprinting evaluation of four varieties of mango reported four phenolic compounds. Mostly the ripened stage had more amounts of phenolics.

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CONFLICT OF INTEREST: There is no conflict of interest.

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