

## Descriptive analytical study based on Profiling, morphological, pomological and pharmacological traits to identify the genotypes of the promising mango [*Mangifera indica* L.]

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### Abstract

The mango, or *Mangifera indica* L., is one of the most well-known tropical fruits in the world and a member of the Anacardiaceae family. Additionally, the mango fruit is rich in a number of phytonutrients, vitamins, carotenoids, omega-3 and omega-6 fatty acids, polyphenols, amino acids, and nutritional minerals including potassium and copper. Gallic acid is the most prevalent phenol compound in the mango mesocarp, with mangiferin, gallic acid, gallotannins, quercetin, isoquercetin, ellagic acid, and -glucogallin all being found there. There have been claims made about the antiviral, antibacterial, analgesic, anti-inflammatory, and immuno-modulatory properties of *M. indica* extract. A total of 81 trees from 18 different mango (*M. indica*) genotypes were collected and studied and their characters are evaluated. Mango plant extract were collected and used to study about the physicochemical parameters, HPTLC, antioxidant activity and total phenolic compounds were studied.

**Keywords:** Phytonutrients; Genotypes; Antioxidant; Physicochemical parameters; Pomology

### 1. Introduction

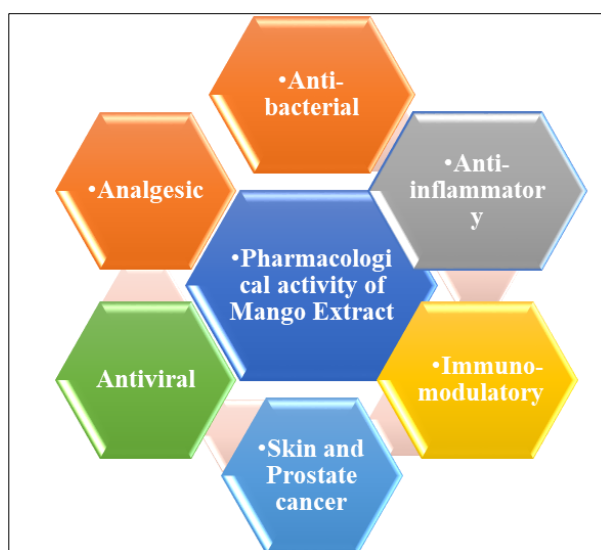
The mango, or *Mangifera indica* L., is one of the most well-known tropical fruits in the world and a member of the Anacardiaceae family. With global output topping 26 million tonnes in 2004, it is one of the most significant fruits marketed worldwide [1]. One of the world's most prized tropical and subtropical fruit crops is the mango. The fact that it is known to as the "King of Fruits" in the tropical world indicates its widespread use and significance [2]. Due to its distinct exotic flavour, taste, and nutritional content, it is widely accepted [3].

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**Figure 1** *Mangifera indica*

Along with the value-added products manufactured from it, the mango fruit's high quantities of vitamins, minerals, and fibre contribute to its rising popularity. As a result, the fruit generates income from both domestic sales and overseas earnings upon export [4]. According to the USDA's database of nutrients, mangoes are a great source of prebiotic dietary fibres, folate, and vitamins A, C, B6, and B9. Mangoes were produced in the largest quantities of any tropical fruit in the world. India is the world's top producer of mangoes, according to data from [5]. Additionally, the mango fruit is rich in a number of phytonutrients, vitamins, carotenoids, omega-3 and omega-6 fatty acids, polyphenols, amino acids, and nutritional minerals including potassium and copper. Gallic acid is the most prevalent phenol compound in the mango mesocarp, with mangiferin, gallic acid, gallotannins, quercetin, isoquercetin, ellagic acid, and -glucogallin all being found there [6, 7]. Additionally, up to 25 different carotenoids, including provitamin A, lutein, -carotene, and -carotene, that are responsible for the fruit's yellowish hue have been found in the mesocarp fraction [8]. Due to these characteristics, numerous studies have documented their usefulness in preventing skin and prostate cancer [9-11]. Due to its antioxidant effects, it also protects senile individuals from serum oxidative stress [12]. Mangoes' internal and external (Peel and flesh) antiproliferative properties have been observed in breast cancer cell lines [13]. According to Banerjee et al., polyphenolics components inhibited the formation of tumours in breast cancer xenografts in mouse models [14]. Mango extracts have been shown to inhibit or stop some colon and breast cancer cells in an in vitro model, according to the literature [15]. There have been claims made about the antiviral, antibacterial, analgesic, anti-inflammatory, and immuno-modulatory properties of *M. indica* extract [16].



**Figure 2** Pharmacological activity of Mango Extract

Mango has reportedly been found to exhibit a wide range of phenotypic variations resulting from various agroclimatic conditions in various mango growing regions, allopolyploidy, outbreeding, recurrent grafting, and other factors [17]. It still has to be done to thoroughly characterise and adopt for cultivation the significant commercial mango varieties that have been introduced in a number of nations. Additionally, mango cross-pollination may have produced undiscovered new

kinds [18]. As a result, there is a lot of nomenclature uncertainty surrounding mango varieties, with various synonyms being used to describe the same variety. Agronomists are also more interested in how observable morphological and agronomic variants might be utilised for sustainable farming, whereas geneticists and plant breeders are more interested in diversity at the molecular level [19]. Due to their lack of familiarity with the characteristics of the numerous different cultivars of mango that are now grown and available in the nation, farmers are also challenged with the task of identifying cultivars that are productive for their agroecological zones, which results in lower productivity [18, 20]. Exact knowledge of the genetic linkages between the accessions is needed in order to use the conserved germplasm in breeding operations. Avoiding duplication will be made easier with knowledge of the genetic distance between the germplasm accessions, which will also serve to broaden the genetic base of the core collections and, eventually, aid in the preservation of the valuable variety [21].

A straightforward, official, and regulated technique for locating and displaying genetic diversity is morphological characterisation. The presence of fruits is typically necessary for the assessment of morphological diversity in fruit crops. Unfortunately, the fruiting season is short for the majority of fruit crops. Farmers, grafters, nursery managers, and breeders still need to distinguish between kinds at times like rootstock selection and discrimination, or even during artificial pollination, even when it is not fruiting season. Identification of vegetative characteristics that can be employed in the absence of fruits is therefore necessary [18]. On the morphological and pomological characteristics of Iranian mango germplasm, nothing is known. Therefore, the primary goals of the current study project were to describe and assess the morphological and fruit traits of the indigenous popular mango genotypes grown in the Iranian province of Sistan-va-Baluchestan. For managing mango landraces, production, genetic conservation, and further breeding programmes for this crop's sustainable improvement, a characterization, assessment, and documentation system for the studied germplasm will be useful.

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## 2. Material and methods

### 2.1. Plant material

A total of 81 trees from 18 different mango (*M. indica*) genotypes, each with 3–10 replications, were examined from eight different regions of India such as Uttarakhand especially in the region near Pantnagar (Department of Entomology, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India), Himachal, Delhi, Haryana, Madhya Pradesh, Rajasthan, Uttar Pradesh including Kashmir [located at 33°27'78"N latitude, 75°34'12"E longitude, and 1,585 m height above sea level].

### 2.2. The characters evaluated

To explore phenotypic diversity among the genotypes, 33 morphological and pomological characteristics were utilised in total [Table 1]. 50 replications of each genotype's leaves and fruits were used for the morphological and pomological analyses. A digital calliper was used to measure the measurements of the leaf, fruit, stone, and seed. A 0.01 g precision electronic balance was used to measure the weight of the fruit, stone, and seed. Using rating and coding [Table 2] and the mango guidelines description [22], the remaining characters were qualitatively evaluated.

### 2.3. Statistical analysis

Analysis of variance (ANOVA) was used to assess genotype variation based on attributes evaluated with SAS software [23]. Pearson correlation coefficients were used to find straightforward connections between attributes [24]. With the use of the SPSS statistical package, principal component analysis (PCA) was performed to look into the relationship between genotypes and identify the key features that contribute to genotype segregation. Using PAST software, hierarchical cluster analysis (HCA) was carried out using Ward's approach and the Euclidean coefficient [25]. PAST software was used to make a scatter plot using the first and second principal components [PC1/PC2]. Additionally, through multiple regression analysis [MRA] utilising the "linear stepwise" approach and SPSS software, independent variables influencing the fruit weight as a dependent trait were identified.

The mango fruit has a distinctive colour and flavour thanks to the presence of numerous phytochemical components. These phytochemicals, which are primarily phenolics, can be found in several parts of a tree, including the fruit, kernel or stone, leaves, and bark [26]. Depending on the cultivar, cultivar region, and maturation stage, the chemical makeup of the various mango portions changes. Using catechol, gallic acid, 3-methylcatechol, protocatechuic acid, and pyrogallol as substrates, [27] found that the crude extracts of the cultivar Ataulfo had polyphenol oxidase activity. 20 polyphenols, including caffeic acid, p-coumaric acid, gallic acid, gallotannin, kaempferol (hexose), mangiferin, protocatechuic acid, quercetin 3- ara-glc (peltatoside), and six quercetin derivatives were found in the mango pure.

**Table 1** *Mangifera indica* analysis of variance genotype traits showing pomological characteristics

No.	Traits	Unit	Min.	Max.	Mean	SD	CV (%)
1.	Tree growth habit	Code	1	5	2.80	0.98	35.65
2.	Tree height	Code	1	7	4.25	1.87	43.92
3.	Crown shape	Code	1	7	5.38	2.09	37.52
4.	Crown diameter	Code	1	5	4.38	1.14	25.68
5.	Branch density	Code	1	5	3.98	1.34	31.97
6.	Shoot color	Code	1	7	4.52	1.62	43.08
7.	Trunk type	Code	1	3	1.28	0.69	53.47
8.	Harvest date	Code	1	16	8.56	3.65	46.24
9.	Yield	Code	1	6	3.17	1.57	47.18
10.	Leaf texture	Code	1	3	1.45	0.95	56.98
11.	Leaf blade shape	Code	1	9	3.74	2.55	73.10
12.	Leaf blade length	mm	91.47	319.82	192.37	54.69	27.42
13.	Leaf base shape	Code	1	5	2.98	0.84	24.52
14.	Petiole color	Code	1	5	2.74	1.58	53.74
15.	Petiole thickness	mm	1.45	3.71	2.50	0.49	20.01
16.	Petiole length	mm	9.12	54.52	27.95	11.42	38.82
17.	Fruit shape	Code	1	7	5.48	2.06	36.58
18.	Fruit diameter	mm	38.21	95.12	64.21	11.98	18.94
19.	Fruit length	mm	45.87	142.97	95.87	21.05	21.68
20.	Fruit weight	g	44.64	427.51	179.34	93.78	52.45
21.	Fruit pedicel width	mm	1.84	4.98	2.96	0.61	20.14
22.	Fruit pulp thickness	mm	6.14	30.12	18.24	4.58	25.98
23.	Pulp color of ripe fruit	Code	1	7	5.84	2.01	34.64
24.	Pulp juiciness	Code	1	5	3.98	1.42	32.40
25.	Eating quality	Code	1	5	3.64	1.78	43.58
26.	Stone width	mm	21.45	63.45	35.30	6.15	17.52
27.	Stone weight	g	5.48	61.87	33.24	11.54	36.14
28.	Stone length	mm	37.45	120.12	78.14	17.64	22.96
29.	Stone thickness	mm	4.14	28.14	19.84	3.54	18.64
30.	Type of embryonic	Code	1	3	1.54	0.41	35.17
31.	Seed shape	Code	1	3	2.45	1.02	40.18
32.	Seed thickness	mm	1.85	24.58	16.87	3.84	21.48
33.	Seed length	mm	19.20	93.14	62.78	15.16	23.34
34.	Seed weight	g	1.52	39.19	18.47	6.74	38.98
35.	Seed width	mm	7.54	50.14	29.14	6.28	22.14

The mango fruit pulp has been found to co-occur with the phytochemicals homo-, iso-, and mangiferin. One of the main processed products made from mango fruits is the pulp. It contains a lot of phytochemicals. There are several polyphenols detected in mango pulp, including glucogallin, ellagic acid, gallic acids, gallotannins, isoquercetin, mangiferin, and quercetin. Gallic acid, gallic acid methyl ester, and gallic acid propyl ester were also discovered in the pulp. Gallic acid, which is found in gallotannins, and other phenolic acids, which are produced by the oxidation of galloyl residues in ellagitannins, are the two types of phenolic acids present in mango fruits. Many phytochemical levels and vitamin C content of mango fruits are thought to be increased by artificial fruit ripening [28]. Caffeic acid, cinnamic acid, and ferulic acid are the additional phenolic acids found in modest amounts in mango pulp. Ramirez et al. (2014) identified 21 phenolic compounds, the majority of which were homo-mangiferin and mangiferin gallate, which were both found in the pulp of both cultivars and both the peel and pulp, respectively, while dimethyl mangiferin was only discovered in the pulp of the Tommy Atkins mango [29]. Mango peel, a significant by-product of the mango processing industries, makes up around 15–25% of the weight of the mango fruit overall. Polyphenols, carotenoids, dietary fibre, and vitamins E and C are reported to be present in significant amounts. The polyphenolic components of mango peels include kaempferol, ellagic acid, mangiferin, quercetin, rhamnetin, and their connected conjugates. Mangiferin was found in abundance in the mango peel and seed kernel. Gallic, protocatechuic, gentisic, and syringic acids are the phenolic acids linked to the mango peel extract in acetone. To Unripe mango peels included more polyphenol components, whereas ripe mango peels have relatively larger levels of carotenoids and anthocyanins. In order to create functional food items, it is advised to use the mango peel as a component because it has higher levels of total phenols, flavonoids, gallic acid, mangiferin, and antioxidant capacity than the mango pulp [30]. Gallotannins, benzophenone derivatives, and flavonol O- and xanthone C-glycosides are all abundant in mango peel. In the mango peel, there were found to be 18 gallotannins and 5 benzophenone derivatives, which were probably galloylated maclurin and iriflophenone glucosides. According to research rutin was a minor flavonoid among the binding flavonoids found in mango peel, with kaempferol and quercetin being the predominant ones [31]. Microelements including Se, Cu, and Zn are found in large quantities in the mango kernel and stone, which is a good source of phenolic compounds. The high levels of polyphenols, sesquiterpenoids, phytosterols, and the microelements Se, Cu, and Zn in mango kernels are what give them their anti-oxidant properties. Mango kernel antioxidants are a possible source of natural antioxidants that could replace chemically manufactured antioxidants in the food business [32]. Tannin, gallic acid, coumarin, caffeic acid, vanillin, mangiferin, ferulic acid, cinnamic acid, and several other polyphenolic chemicals can all be found in the mango kernel. The amount of phenolics in the mango kernel extract was found to be considerable, with 85.7 percent of them being methyl gallate [33].

**Table 2** List of plant parts with their phytoconstituents and pharmlogical activity

Mango Part	Phytoconstituents	Pharmacological activity
Juice of pulp	Carotenoids, terpenoids, polyphenol	Inhibit free radical production and neoplastic
Leaf extract	Quercetin, kampferol	Anti-analgesic, antimicrobial & anti-inflammatory
Seed extract	Polyphenols, fatty acids and flavanoids	Antibacterial against gram+ve and gram-ve bacteria
Stem bark	Mangiferin, terpenoids, tannins	Anti-plasmodial
Mango peel	Carotenoids, dietary fibre and polyphenols	Antioxidant and anti-inflammatory

The mango leaves and bark have extremely high concentrations of phenolic compounds from diverse therapeutic uses. Mangiferin and homo-mangiferin were both first discovered in mango leaves and bark, respectively [34]. Protocatechuic acid, catechin, mangiferin, alanine, glycine, 4-aminobutyric acid, kinic acid, shikimic acid, and the tetra cyclic triterpenoids were all said to be present in mango bark. Gallic acid, xanthonoids, mangiferin, and mangiferin 6-O-gallate were among the polyphenols that the mango leaves were said to have in large concentrations in the leaves [35]. The leaves of the Alphonso mango contained the phenolic chemical pyrogallol, which is thought to be poisonous [36]. The secondary metabolites found in the mango leaves include alkaloids, flavonoids, tannins, saponins, total phenol, and cardiac glycosides. Due to the antioxidant capabilities of total phenols, which are present in greater amounts in its leaf extract, this substance may be used to treat a variety of ailments [37]. The primary flavonoids in mango are quercetin and catechin. Pigment accumulation in the mango fruit is cultivar dependent. However, mango has a high content of carotenoids in mesocarp tissue responsible for the intense yellow color [38]. 25 mango Pulp was used to separate several carotenoids, with carotene having the highest concentration and being responsible for the majority of mango cultivars' yellow or orange coloration.

### 3. Results and discussion

The Botanist at the Centre of Medicinal Plants Research in Homoeopathy (CMPRH), Emerald, Tamil Nadu, and Professors & scholars of various university/Institute gathered the stem bark of Mang. ind., and the CMPRH botanist verified its authenticity. Ascorbic acid, rutin hydrate, and mangiferin (C<sub>19</sub>H<sub>18</sub>O<sub>11</sub>) were acquired from Merck, sodium hydroxide pellets, sodium nitrite, and aluminium chloride anhydrous powder from Sigma-Aldrich (India), (Merck Ltd.'s analytical grade pure solvents included ethyl acetate, glacial acetic acid, formic acid, chloroform, and methanol. Germany's M/s Merck Ltd. provided the phenol reagent, while ELGA Lab Water provided the HPLC water.

#### 3.1. Physicochemical study

To determine the amount of loss during drying at 105°C, stem bark was dried, ground into a coarse powder, and then tested. Standard techniques were used to measure total ash, acid-insoluble ash, physicochemical parameters, and UV spectroscopic studies.

**Table 3** Physicochemical parameters

Parameters	Quantitative values
Loss on drying at 105°C	Not more than 24.3% w/w
Total ash value	Not more than 7.34% w/w
Acid-insoluble ash	Not more than 0.78% w/w
Alcohol-soluble extractive value	Not less than 15.14% w/w
Water-soluble extractive value	Not less than 18.13% w/w

#### 3.2. Preparation of mother tincture

To create 1000 mL of mother tincture using the percolation method, 100 g of coarsely powdered bark was combined with 670 mL of strong alcohol and 360 mL of water (as per the Homoeopathic Pharmacopeia of India, Volume VII) [39].

#### 3.3. Preparation of standard solution

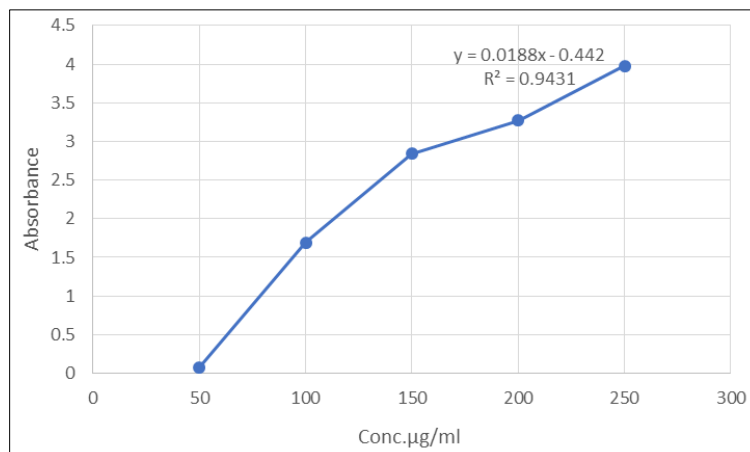
The stock solution was made by precisely weighing 10 mg in a 10 mL volumetric flask, then diluting it with methanol. Standard has a concentration of 1000 µg in 1000 µL.

#### 3.4. HPTLC studies

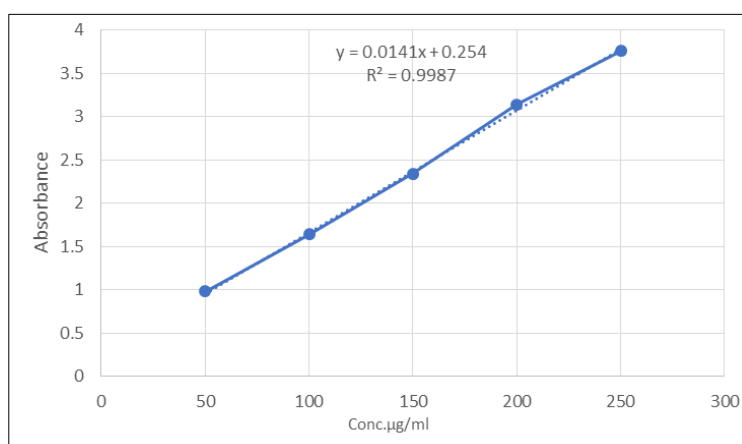
The HPTLC CAMAG Linomat V with vision CATS software was utilised as the analytical tool for HPTLC analysis. Following HPTLC analysis using various solvent systems, mangiferin was identified in in-house sample (B) (track 7-9) and market sample (C and D) (track 10-14) using reference standard mangiferin (A) (track 1-6) and ethyl acetate: glacial acetic acid: formic acid: water (7:1:1:1, v/v/v/v) [40,41]. In a 50 mL beaker, 25 mL of mother tincture were consumed. The solution was evaporated over a water bath and extracted three times with 20 mL chloroform to get rid of the ethanol. Extract of chloroform was mixed and concentrated to a volume of 2 mL. On a silica gel 60 F254 pre-coated plate, HPTLC was performed on a chloroform extract of mother tincture and reference standard mangiferin using a mobile phase of ethyl acetate, glacial acetic acid, formic acid, and water (7:1:1:1, v/v/v/v). As a sample applicator, a Camag Linomat V was utilised, and a Camag Twin Trough glass chamber (20\*10) was utilised for the formation of the mobile phase. The CAMAG microliter syringe was used to spot the concentrated chloroform extract, which had a band width of 8.0 mm. On a silica gel 60 F254 pre-coated plate (20\*10 cm plate from Merck), spots were created using a sampling machine, and the solvent front was run up to a height of 70 mm. The CAMAG TCL Scanner and VISION CATS programme carried out densitometric scanning at 254 nm and 366 nm. With the aid of win aspect software, UV spectrophotometer SPECORD 200 Plus UV analysis was carried out (Analytik Jena, Germany). The samples were made with ethanol at a volume-to-volume ratio of 1:99. (mother tincture: ethanol). Samples, a reference, and a spectrophotometer with a range of 190–600 nm were placed in cuvettes. Cuvettes were cleaned with ethanol prior to analysis, and win Aspect software was used for the UV analysis. In-house samples were prepared for UV analysis by combining one part mother tincture with 99 parts absolute alcohol (1:99), then filtering the mixture via a membrane filter prior to UV examination. Max 316 nm and 366 nm were used to measure the analysis peak [Figure 1].

### 3.5. Antioxidant study

#### 3.5.1. Determination of total phenolic content (TPC)



**Figure 3** Antioxidant property of Ascorbic acid



**Figure 4** Antioxidant property of Rutin hydrate

The Folin-reagent, Ciocalteu's which measures TPC in terms of ascorbic acid equivalent, was used to calculate the TPC of the extracts. Different amounts of ascorbic acid (0.26615-8.517 mM) were generated, measured at 742 nm, and a calibration curve was displayed as absorbance against concentration. Ascorbic acid was employed as the standard chemical. Ascorbic acid served as the benchmark for TPC determination. 50 ml of the mother tincture, 5 ml of 10% Folin- (a Ciocalteu's phenol reagent), and 4 ml of sodium carbonate were combined. The mixture was left to stand in the dark for one hour. The yellow tint turned blue after one hour. A UV-visible spectrophotometer was used to test the solutions' absorbance at their maximum wavelength of 742 nm (UV Spectrophotometer SPECORD 200 Plus Analytik Jena, Germany). To create a calibration curve from which the phenolic content of mother tincture was calculated in terms of its ascorbic acid equivalent, ascorbic acid (0.26615-8.517 mM) was employed as the standard. The TPC was computed using the calibration curve [Figure 3], and the ascorbic acid equivalents of the TPC for the in-house mother tincture and two market samples were determined in the final results [42]. Calculating the content of all flavonoids (TFC) Using a colorimetric assay using aluminium chloride, TFC was calculated. The calibration curve was constructed as absorbance against concentration using rutin hydrate as the standard chemical. Different quantities of rutin (15.625-250 g/mL) were generated and evaluated at 508 nm. An aliquot of 1 mL of the mother tincture, 4 mL of distilled water, and 300 l (5%) sodium nitrite are used to determine the TFC. After 5 minutes, add 300 l of 10% aluminium chloride, followed by 2 ml of methanol, 2 ml of 1M sodium hydroxide, and 2.4 ml of distilled water to bring the total volume to 10 ml. After giving the mixture a thorough shake, it was left to cool to room temperature in the dark. Yellow turns pink in the ensuing combination. A spectrophotometer was used to measure the reaction mixture's absorbance at max. 508nm. Rutin hydrate standard solutions of 15.625-250 g/mL of each standard were used to create a standard calibration curve and were processed in the same way as the samples mentioned above [Figure 4]. From the curve, the TFC of each mother

tincture produced in-house and two market samples were computed, and the results were reported as the rutin hydrate equivalents [43].

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#### 4. Conclusion

In this research paper we studied about the mango the genotype and prototype study of the different mango species and studied different descriptors. As we know mango was rich in nutrients, vitamins and various different phytoconstituents are present in different parts of mango which shows different pharmacological activities. We also studied HPTLC, total phenolic content and antioxidant activity of mango plant. We compared the rutin hydrate a phytoconstituents shows equivalent antioxidant activity when it is compared to the ascorbic acid and also shows TFC equivalent to the marketed formulation. Hence, we can say that each part of mango is beneficial to the human species.

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#### Compliance with ethical standards

##### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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