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Original Article

HPTLC FINGER PRINT ANALYSIS AND PHYTOCHEMICAL INVESTIGATION OF *MORINDA TINCTORIA* ROXB LEAF EXTRACTS BY HPLC AND GS MS

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ABSTRACT

Objective: The present study was conducted to investigate the qualitative and quantitative screening of phytochemicals, and characterization by HPTLC finger print analysis, HPLC and GC MS substantiation for the presence of scopoletin and rutin in methanolic leaf extracted of *Morinda tinctoria* Roxb. (MEMT).

Methods: The Qualitative and Quantitative Phytochemical analyses were carried out as per the standard protocols. The MEMT characterization was performed by High-Performance Thin Layer Chromatography (HPTLC), High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass spectrometry (GC-MS).

Results: The preliminary phytochemical screening of methanolic extracts revealed the presence of alkaloids, flavonoids, phenolics, terpenoids, saponins, tannins, phlobatanin, quinones, coumarins, cardiac glycosides and proteins. The quantitative phytochemical screenings in terms of total alkaloids, total flavonoids, total phenolic, total proteins and DPPH scavenging activity of MEMT were found to be 13 ± 0.034 mg/g, 0.06±0.003 mg/g, 0.08 ±0.012 mg/g, 0.29 ± 0.071 mg/g and 62.12 ± 0.250 %. From the HPTLC results, the $R_f$values of scopoletin and rutin were calculated as 0.55 and 0.24. Scopoletin and Rutin content of MEMT was 1.58 and 1.88 % w/w of air dried to extract by HPLC. GC-MS analysis of the MEMT showed the presence of compounds like scopolamine, a secondary metabolite and Malvidin-3, 5-diglucoside, an anthocyanin and a glycoside, as main constituents.

Conclusion: From our results, we found that the proposed HPLC method is fast, discriminating, requires trouble-free sample preparation procedure, and regarded as a good method for quantification in different plant extracts and for regular quality control of herbal formulations.

Keywords: *Morinda tinctoria*, Phytochemical screening, HPTLC, HPLC, GC-MS.

INTRODUCTION

Plants are considered as an archetypal source for a large number of instant phytochemicals, and they have long been used as the sources of medicines. The majority of species of Morinda genus were reported for a wide range of health disorders and anticancer activities in Indian Pharmacopoeia [1]. For instance, *Morinda tinctoria* also known as Indian mulberry is a well-known member of the Rubiaceae family, it is commercially known as Nunaa, and it is native to unfarmed lands of tropical countries and considered as an essential folklore medicine. It is just an evergreen shrub or small tree growing to 5-10 m tall. The leaves are 15-25 cm long, oblong to lanceolate. The flowers are tubular, white, scented, about 2 cm long. The fruit is a green syncarp, 2-2.5 cm diameter as showed in Fig. 1.

Morinda tinctoria leaves contain a set of pharmaceutically important phytochemicals like octoanic acid, potassium, vitamin-C, terpenoids, scopoletin, flavones, glycosides, lineoleic acid, anthraquinones, morindone, rubiadin and alizarin[2]. Anthraquinones are reckoned as one of the major constituents of the Morinda genus, showing antitumor promoting, antioxidant, anti-inflammatory, purgative and astringent activities[3]. This plant serves an excellent medicine against arthritis, diarrhoea, viral infection, astringent, gastric ulcer, liver diseases and diabetes [4].

There is greater demand for Morinda species fruit extracts for different kinds of illness such as arthritis, cancer, gastric ulcer and other heart disease treatment [5]. The leaves of this plant have been investigated for various medicinal properties like cyto protective [6], antimicrobial and anti-inflammatory[5], anticonvulsant [7], macro vertebra colonization [8], antimicrobial[9], *in-vitro* antioxidant activity[10] and removal of ammonia from polluted waters[11]. The fruit extracts have reportedly wound healing [12], anti-hyperglycemic and anti - diabetic [13], anti-inflammatory [14] properties.

Fig. 1: Macroscopic Investigation of *Morinda tinctoria*. Roxb A. Whole Plant B. Leaves C. Flower D. Fruit

Although many researchers and studies have found that synthetic compounds that are very helpful as radio protectors in lab. But these have failed to comply with the transition to human applications due to toxicity and increasing risk of side effects[15]. However, these compounds produced serious side effects and are toxic at the doses needed for radioprotection. To this problem, natural sources, especially edible medicinal plants/herbs might provide an ideal solution as these are regarded as non-toxic even at higher concentrations. In view of low cost, easy accessibility and fewer toxic effects, there is a growing interest on ethnos medicines even among the common people[16]. Since plants have rich sources of polyphenols like flavonoids, coumarins and anthocyanin's etc. Flavonoids scavenge free radicals generated during radiation exposure and protect the endogenous defense antioxidant enzyme

system[17]. Earlier researchers have reported that the Rutin (quercetin-3-rhamnosyl glucoside)[18] is a flavonoid glycoside possess the anti-bacterial [19], anti-inflammatory, antiulcer, antispasmodic [20] and hepatoprotective activity [21].

Scopoletin (6-methoxy-7-hydroxycoumarin)[22]coumarin derivative possesses the anti-mutagenic/anti-carcinogenic activity [17,23], Anti-Gastric Ulcer [24] anti-inflammatory, hypouricemic, and antioxidant activities [25].

The present study was conducted to investigate the qualitative and quantitative screening of phyto chemicals, and characterization by HPTLC finger print analysis, HPLC and GC MS substantiation for the presence of scopoletin and rutin in methanolic leaf extracts of *Morinda tinctoria* Roxb. (MEMT).

MATERIALS AND METHODS

Collection of *Morinda tinctoria* roxb. leaves

Fresh leaves of *Morinda tinctoria* Roxb. Plant of the same age group from a single population was collected from Potheri Forest(12°49'09.9"N 80°03'32.8"E), in the outskirts of SRM University Campus Chennai, Tamil Nadu, India during October, 2013. The herbarium was prepared for authentication (Ref. SRM/CENR/PTC/2013/10) and the taxonomic identifications were done by Dr. Jayaraman, Professor, Department of Botany, Madras Christian College, Tambaram, Chennai, Tamil Nadu. The specimens were deposited in the department herbarium for future reference. The taxonomy of the plant was described in table 1 and the picture of the plant was shown in fig. 1.

Table 1: Taxonomy classification of *Morinda tinctoria roxb*

Kingdom	Plantae
Subkingdom	Tracheobionta
Super division	Spermatophyta
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Order	Rubiales
Family	Rubiaceae
Genus	*Morinda*
Species	*Tinctoria*

Preparation of *Morinda tinctoria* Roxb

Morinda tinctoria. Roxb leaves were initially washed with distilled water to remove the dirt's and other extraneous matter and washed with a mild soap solution and rinsed thrice with distilled water. The leaves were shade dried for two weeks and finely powdered and sieved using 20 µ mesh sieve. The finely sieved leaf powders were soaked in methanol (1:20) for 72 h with occasional shaking, filtered using whatman filter paper No. 1 and concentrated by the rotary vacuum pump to get the solid mass. The obtained solid mass was stored in an airtight container to protect from sunlight for further use.

Qualitative phytochemical analysis

The qualitative phytochemical analysis of *Morindatinctoria* Roxb., extracts were performed following the methods described in our earlier works [26,27]. To determine the presence of alkaloids (Mayer's, Wagner, Dragendorff's test), flavonoids (alkaline reagent, Shinoda), phenolics (lead acetate, alkaline reagent test, Ferric chloride method), terpenoids (Salkowski test), saponins (foam test), tannins (Ferric chloride method), Phlobatanin (HCl method), Quinones (Sodium Hydroxide test), Coumarins (Sodium Hydroxide test), Cardiac glycosides (Keller-Killani test), Proteins (Lowry's method). The results were qualitatively expressed as positive (+) or negative (−) [28]. The chemicals used for the study were purchased from Sigma-Aldrich (Chennai, India).

Quantitative phytochemical analysis

Estimation of total phenolic content

The total phenolic content of the extract was evaluated using an adapted Folin-Ciocalteu colorimetric method. About 200 µl of 10% (v/v) Folin-Ciocalteu reagent were mixed with 100µl of aqueous extracts in the phosphate buffer (75 mM, pH 7.0). Gallic acid was used as a positive control and phosphate buffer as a negative control and the absorbance was measured at 765 nm after 30 minutes of incubation using UV-Vis 3000+ double beam spectrophotometer (Lab India, Maharashtra, India).

A standard curve using gallic acid concentrations ranging from 0.05 to 0.5 mM was constructed, and the results were expressed as mg/g of gallic acid equivalents (GAE) of dried weight of the leaves[29]. All the experiments were carried out in triplicate, and the results were averaged to express as mean ± SD.

Estimation of total flavonoids

The aluminum chloride colorimetric method was used with some modifications to determine the flavonoid content using quercetin as standard (1 mg/ml).1 ml of plant extract was mixed with 3 ml of methanol, 0.2 ml of 10% aluminum chloride, 0.2 ml of 1M potassium acetate and 5.6 ml of distilled water and kept at room temperature for 30 minutes, and the absorbance was measured at 415 nm. All the tests were performed in triplicates. The flavonoid content was reported inquercetin equivalent (mg/g of the leaf extracts)[30].

Estimation of alkaloids

To 5g of the sample, 200 ml of 10% acetic acid in ethanol was added and allowed to stand for 4 minutes, filtered and the extract was concentrated on a water bath to be one-quarter of the original volume. The concentrated ammonium hydroxide was hereby added drop wise to the extract until the precipitation was completed. The entire solution was allowed to settle, and the precipitate was collected and washed with diluted ammonium hydroxide; filtered and the residue alkaloid precipitate was dried and weighed[31].

Estimation of protein

The protein was quantitatively examined by Lowry's method using bovine serum albumin (BSA) as a working standard and distilled water as the blank. The absorbance of developed blue color was measured at 660 nm[32].

DPPH radical scavenging assay

The method of Blios was utilized for the determination of scavenging activity of the DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) free radical. The reaction mixture (DPPH and extract) was vortexed, incubated at room temperature for 30 minutes and its absorbance was measured at 517 nm. The scavenging ability of the plant extracts was calculated using the following equation 1:

$$DPPH\ Scavenging\ activity(\%) = \frac{[ABS_{Control} - ABS_{sample}]}{ABS_{Control}} \times 100 \quad \text{-- (1)}$$

Where, $ABS_{control}$ is the absorbance of DPPH without sample; ABS_{sample} is the absorbance of DPPH with sample[33].

HPTLC fingerprint analysis of MEMT

The High-Performance Thin Layer Chromatography analysis of plant extracts is described in our earlier research[28,31,34]. HPTLC is a planar chromatography where the separation of the sample components is achieved on high-performance layers with detection and acquisition using an advanced work station. Camag HPTLC System, equipped with a Linomat V with Camag 100µl syringe sample applicator, a twin chamber tank, a model Camag Twin through glass chamber (20 × 10), Thin Layer Chromatography (TLC) scanner and Camag TLC scanner III software Win Cats 4.03 version were used in the study. 5 and 10 µl of MEMT extracts was applied to L1 and L5 respectively. 10 µl of standard scopoletin with purity of 90 % (1 µg/µl) was applied to L2 and L4 and rutin with purity of 99 % (1 µg/µl) was applied in L3 in TLC Aluminum sheets (10 cm× 10 cm) of silica gel G60 F 254.

The plates were developed with up to 80 mm under chamber saturation conditions. After air drying the solvent, the plates were scanned using scanner III at 366 nm wavelengths in absorbance mode[34].

HPLC Screening of MEMT

About 100mg of the MEMT extracts was dissolved in methanol and properly diluted to obtain a concentration of 2.5 mg/ml. The prepared solution was sonicated for degassing and shortly passed through a vacuum filter containing whatman filter paper of pore size 0.45 µm to acquire a clear solution. Standards of scopoletin and rutin were prepared in the same manner like sample solution the concentrations used for scopoletin was 100 µg/ml and for rutin was 20 µg/mL. The MEMT and the standard solution were subjected to HPLC separately. An isocratic HPLC (Shimadzu HPLC Class VP series) with two LC- 0 AT VP pumps (Shimadzu), a variable wave length programmable photo diode array detector SPD-M10A VP (Shimadzu), a CTO- 10AS VP column oven (Shimadzu), an SCL-10A VP system controller (Shimadzu), a reverse phase Luna® 5 µm C18 (2) and Phenomenex column (250 mm X 4. 6 mm) was used. The mobile phase constituent methanol: water (0.05% Formic Acid) was filtered through a 0.2 µm membrane filters before use and pumped from the solvent reservoir at a flow rate of 1.0 ml/min. The column temperature was maintained at 27°C. About 20 µl of the respective sample was injected by using a Rheodyne syringe (Model 7202. Hamilton) and the HPLC peaks were observed at 220 and 254 nM. Further analysis was also carried out to compare the HPLC chromatogram of MEMT against standard compound's Rutin and scopoletin separately[35].

GC-MS analysis

GC-MS analysis was carried out using GC Clarus 600 Perkin Elmer system interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-35ms fused silica capillary column (30 X 0.25 mm ID X 0.25 mm film thickness, composed of 5% Phenyl, 95% Dimethyl Polysiloxane), operating in electron impact mode at 70 eV, helium (99.99%) was used as carrier gas at a constant flow of 1 mL/min, injector temperature 200°C; ion-source temperature 150°C. The oven was programmed with initial temperature 50°C for 10 min, with an increase of 7°C/min, to 280°C hold for 10 Min. Mass spectra was taken at 70 eV, a scan interval of 0.2 s and fragments were scanned from 50 to 550 Da [36].

Statistical analysis

The experimental results were performed in triplicate, and data obtained were statistically analyzed using two-way analysis of variance (ANOVA) followed by Bonferronipost test for multiple comparisons between pairs using graph pad prism 5.0. Results are reported as mean values ± S. D and differences were considered as highly significant when $P \leq 0.001$.

RESULTS AND DISCUSSION

Qualitative and quantitative screening of phytochemicals

Preliminary phytochemical screening results of methanolic leaf extract of *Morinda tinctoria* Roxb.(MEMT) showed the presence ofmany phytochemicals such as alkaloids, flavonoids, phenolics, terpenoids, saponins, tannins, phlobatanin, quinones, coumarins, cardiac glycosides and proteins (Table 2).

Table 2: Qualitative phytochemical screening of leaf extract of *Morinda tinctoria*

S. No.	Phytochemicals Compounds	*M. tinctoria*(Methanolic Extract)
1.	Tannin	+
2.	Phlobatanin	+
3.	Saponins	+
4.	Flavonoids	+
5.	Alkaloids	+
6.	Quinones	-
7.	Coumarin	+
8.	Terpenoids	+
9.	Cardiac glycosides	+
10.	Phenol	+
11.	Proteins	+
12.	Glycosides	+

As reported in earlier studies, flavonoids and phenolic compounds exhibited a wide range of biological activities like antioxidant and lipid peroxidation inhibition properties [37,38].

Note: + Present, - Absent

As tabulated in table 3, the total flavonoids were 0.06±0.003mg/g, total phenol was 0.08 ±0.012 mg/g, and alkaloidwere13 ± 0.034 mg/g and protein 0.29 ± 0.071mg/g was present in MEMT. The flavonoids and phenolic compounds are primarily responsible for a broad range of biological activities like antioxidant and lipid peroxidation inhibition properties [39].

Table 3: Qualitative phytochemical constituents of *Morinda tinctoria*

S. No.	Phytochemical	(mg/g)
1.	Phenol	0.08 ± 0.012
2.	Flavonoids	0.06 ± 0.003
3.	Alkaloid	13 ± 0.034
4.	Protein	0.29 ± 0.071
5.	Free radical scavenging (%)	62.12 ± 0.250

All the values represented are the averages of three observations. Data presented as the mean ± standard deviation

DPPH radical scavenging activity

The DPPH radical is considered as moderately stable, which has been extensively used to test the capability of a plant extract to act as a free-radical scavenger or hydrogen contributor and thus to appraise its antioxidant activity [40]. Plant phenolic compounds are reported as dominant in-vitro antioxidants due to their capability to provide hydrogen or electrons and to form stable radical intermediates[41]. MEMT suppressed DPPH free radical in a dose-dependent manner. As shown in Figure2, the DPPH suppressing activity of MEMT increased with increasing concentration of the MEMT due to the neutralization of free radicals by transfer of an electron or hydrogen atom. The MEMT showed antioxidant activity in the DPPH assay with immense free radical scavenging activity of about 62.12 ± 0.250 % (Table 2). Fig. 2 shows the inhibitory potential of the MEMT compared with Standard ascorbic acid, the well-known antioxidant.

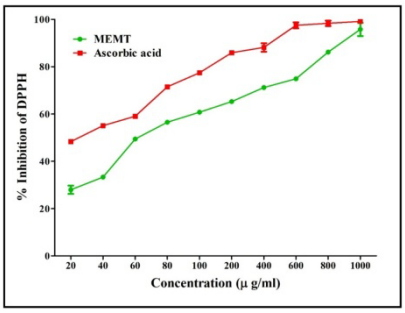


Fig. 2: Comparison of DPPH radical scavenging capacity of MEMT with standard ascorbic acid

HPTLC fingerprint analysis of MEMT

After continuous trials by using different mobile phases for the separation of methanolic leaf extract of *Morinda tinctoria* Roxb. (MEMT) by HPTLC, the desired resolution of scopoletin and rutin with reproducible peaks were succeeded using Chloroform: Methanol (9.5:0.5) as the mobile phase using 5.0 µl and 10 µl volumes of MEMT. The regression data have shown a good linear relationship over the concentration range of 2–20 µl /spot. The linearity of calibration graphs and adherence to the system to Beer's law is validated by high value of correlation coefficient and the SD

for intercept value is noticed to be less than 5%. No significant difference is observed in the slopes of standard curves was observed.

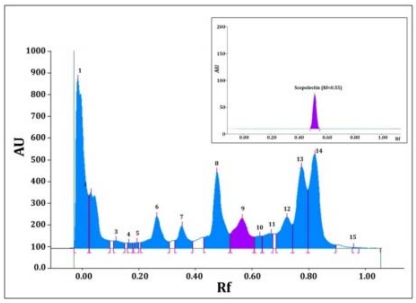


Fig. 3: HPTLC Chromatogram of MEMT and the Insert with in image shows the standard Scopoletin

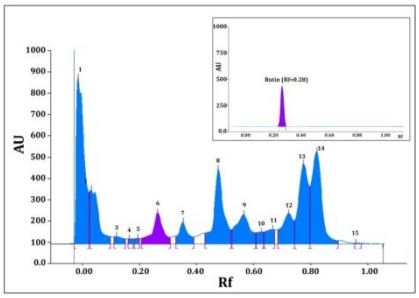


Fig. 4: HPTLC Chromatogram of MEMT and the insert with in the image shows the reference standard Rutin

To ascertain peak purity of MEMT, we compared methanol extract reflectance spectra with standard, which provides test sample purity as shown in fig. 3 and 4. Fig. 3 shows the HPTLC Chromatogram of MEMT, and the Insert within the image shows the standard Scopoletin with Rf value of 0.55. Fig. 4 shows the HPTLC Chromatogram of MEMT, and the Insert within the image shows the standard rutin with Rf value of 0.28. The acquired TLC plate was visualized in CAMAG Visualizer, and many bright color spots observed at 366 nm were represented in the Figure. 5.

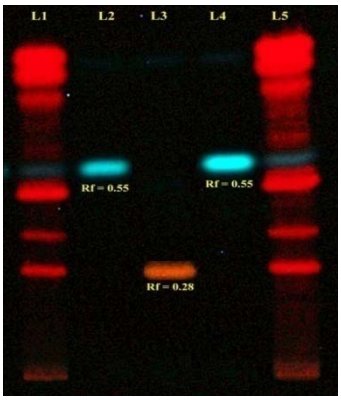


Fig. 5: HPTLC chromatographic plate of MEMT viewed at 366 nm showing the Rutin and Scopoletin

From our results, it was clear that the calibration curve was linear; the correlation coefficient value of 0.99 indicated the good linearity between the area and the concentration (results not shown). The obtained Rf values from the chromatogram matched well with reference standards scopoletin and rutin in the peak display at 10 µl applied volume. Although high-performance thin layer chromatography (HPTLC) has a few limitations, such as the limited developing distance and lower plate efficiency by comparison with HPLC and GC, it is still an effective tool for quality evaluation of medicinal plants due to its simplicity, low cost, and requirement, and it has been successfully utilized to develop the chromatographic fingerprint for medicinal plants. Moreover, the above-mentioned shortcomings can be overcome by separately developing fractions of different polarity on two or several thin layer plates[42]. In summary, the proposed HPTLC method for the simultaneous analysis of scopoletin and rutin from MEMT reported here is very simple, sensitive, economic and suitable for rapid routine quality control analysis[43].

HPLC analysis of MEMT

The HPLC chromatograms are shown in fig. 6 and 7, which depict a good separation of the peaks for all the analytes tested. The qualitative and quantitative content of scopoletin and rutin present in MEMT was calculated by HPLC. The standard compound scopoletin used for HPLC analysis was eluted at retention time (Rt) 7.879 minutes, and the scopoletin in the MEMT used for HPLC also showed a similar retention time (Rt) at 7.803 minutes (Fig. 6).

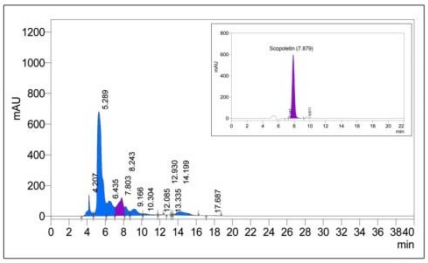


Fig. 6: HPLC chromatogram of MEMT at 220 nm insert within the image reference standard Scopoletin

The standard compound rutin was eluted at 4.885 minutes, and the peak area was compared with the rutin of the MEMT, which showed the peak at 4.946 minutes (Fig. 7) respectively. So the MEMT was quantified for the presence of selected compound's scopoletin and rutin by its retention time (Rt) by HPLC results, which were similar to standard. The scopoletin and rutin content of MEMT was found to be 1.58% w/w and 1.88% w/w of air dried extract. As it can be seen, no interference of peaks is observed in the HPLC chromatogram which establishes high sensitivity of the improved method.

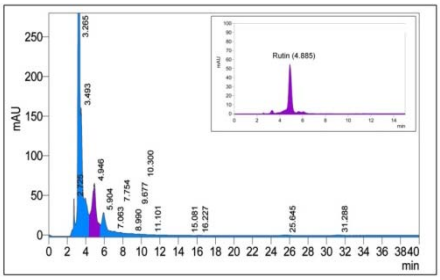


Fig. 7: HPLC chromatogram of MEMT at 254 nm insert within image reference standard Rutin

GC-MS analysis

GC-MS analysis of the MEMT showed the presence of major compounds, scopolamine, Malvidin-3, 5-diglucoside and minor compounds, a tropane alkaloid ecgonine, a triterpenoidoleanoic acid, a monoamine alkaloid cathinone, a xanthanoidmangiferin, a

flavonoid like orientin and mycophenolic acid. The GC-MS chromatogramis illustrated in fig. 8. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The large compound fragments into small compounds giving rise to appearance of peaks at different m/z ratios. This mass spectra are fingerprint of that compound which can be identified from the data library; the identified compounds were listed in table 4. The GC-MS analysis revealed that the MEMT mainly composed of flavonoids, alkaloids and glycosides. Thus this type of GC-MS analyses is the first step towards understanding the nature of active principles in this medicinal plant, and this type of study will be useful for further detailed study. Further investigation may lead to isolation of bio-active compounds, and their structural elucidation and screening of pharmacological activity will be useful for further drug development.

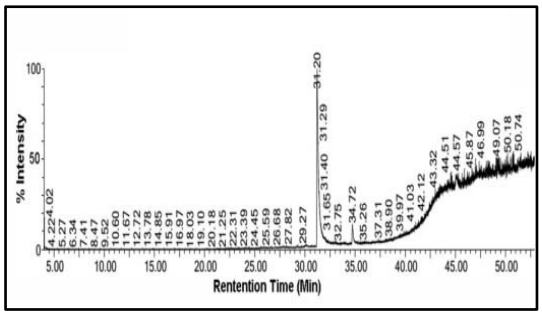
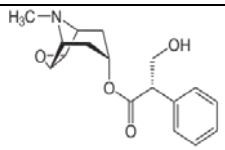
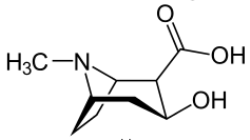
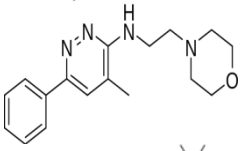
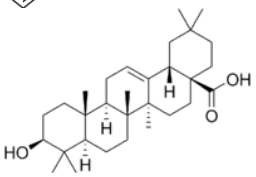
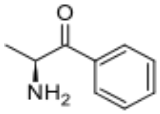
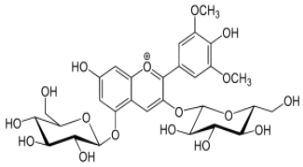
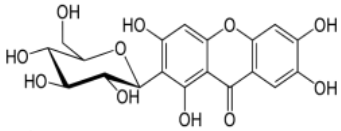
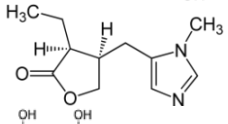
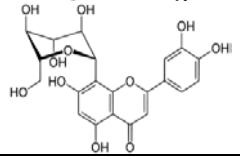
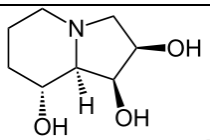
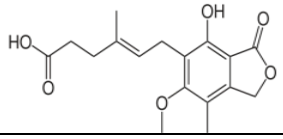


Fig. 8: GC-MS chromatogram of methanolic extracts of *Morinda tinctoria.* Roxb

Table 4: GC-MS profile of the Identified Compounds in *Morinda tinctoria*

S. No.	Retention Time	Compound	Structure	Molecular formula/wt.	Nature and uses
1.	31.214	**Scopolamine**		$C_{17}H_{21}NO_4$ (303.35 g/mol)	Secondary metabolite - Used in the treatment of motion sickness, Postoperative nausea and vomiting.
2.	31.214	**Ecgonine**		$C_9H_{15}NO_3$ (185.22 g/mol)	Tropane alkaloid - it is both a metabolite and precursor
3.	32.054	**Minaprine**		$C_{17}H_{22}N_4O$ (298.383 g/mol)	Heterocyclic organic compound- Used as antidepressant.
4.	43.729	**Oleanoic acid**		$C_{30}H_{48}O_3$ (456.70 g/mol)	Naturally occurring triterpenoid- Used as male Contraceptive
5.	43.729	**Cathinone**		$C_9H_{11}NO$ (149.19 g/mol)	Monoamine alkaloid, cathinone induces the release of dopamine creating a stimulant effect.
6.	44.169	**Malvidin-3,5-diglucoside**		$C_{29}H_{35}ClO_{17}$ (691.0 g/mol)	Anthocyanin- its glycosides also responsible for the color of primroses, and Anticancer
7.	44.169	**Mangiferin**		$C_{19}H_{18}O_{11}$ (422.34 g/mol)	Xanthonoid – it has antioxidant, antimicrobial, gatroprotective and antidiabetic.
8.	44.399	**Pilocarpine**		$C_{11}H_{16}N_2O_2$ (208.25 g/mol)	Parasympathomimetic alkaloid- used to treat xerostomia, used to diagnose cystic fibrosis.
9.	45.024	**Orientin**		$C_{21}H_{20}O_{11}$ (448.38 g/mol)	A chemical flavonoid-like compound. Protect cell structures as well as chromosomes from radiation and oxygen-based damage.

10.	45.104	**Swainsonine**		$C_8H_{15}NO_3$ (173.21 g/ mol)	Indolizidine alkaloid - Appetite suppressant, anti-cancer
11.	48.076	**Mycophenolic acid**		$C_{17}H_{20}O_6$ (320.34 g/mol)	Mycophenolate- Used in the place of anti-proliferative azathioprine, part of immune-suppressants

CONCLUSION

The presence of scopoletin and rutin in *M. citrifolia* has already been reported in the literature, but no works have been reported in *M. tinctoria*. Flavonoids like rutin and scopoletin derivatives appear to be present in view of the characteristic maximum of absorbance shown by MEMT were confirmed by HPTLC. Compound identification was confirmed by comparing their HPLC retention time and mass spectrum with those of standards. Based on our results, it can be confirmed that the bioactive compound's scopoletin and rutin of 1.58% w/w and 1.88% w/w of air dried extracts were present in *Morinda tinctoria* Roxb. The suggested HPLC method in our research is fast, discriminating, requires trouble-free sample preparation procedure, and considered as a suitable method for their quantification in different plant extracts and in regular quality control of herbal formulations. As reported by Somnath et al., 2013 [44] that GC–MS profile can be invoked as biochemical markers in the pharmaceutical industries to identify the authentic mother plants. Similarly, our GC MS results could be invoked as biochemical markers.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest.

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