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Extraction and Phytochemical Screening of *Morinda coreia* Buch-Ham Flower

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ABSTRACT: To evaluate the pharmacognostical and phytochemical screening on flower extracts of *Morinda coreia* and to trace out the bioactive compound which will be responsible for pharmacological activity by chromatography technique. The shade dried flowers of *Morinda coreia* were powdered and extracted with various solvents such as ethanol, ethyl acetate and water by maceration. In qualitative phytochemical screening, the powder and extracts revealed the presence of primary and secondary metabolites such as carbohydrate, protein, flavonoid, saponin, tannin and sterols. The aqueous & ethanolic extracts of *Morinda coreia* showed the presence of quercetin, whereas ethyl acetate extract revealed the presence of gallic acid and rutin by high performance thin layer chromatography (HPTLC). The results suggested that, the flower extracts of *Morinda coreia* has secondary metabolites confirmed by HPTLC, these phytoconstituents can be essential for health to prevent from various diseases.

KEYWORDS: *Morinda coreia* flower extracts,pharmacognostical, phytochemical screening and HPTLC.

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I. INTRODUCTION

Medicinal plants contain different pharmacologically active compounds that act individually to improve health. Since ancient times, many herbs have been potentially used as an alternative remedies for treatment of many infectious diseases. The tribal people cure their ailments by using crude drugs and different parts of plants which are locally available. The traditional systems of medicine are still considered as a great knowledge based in herbal medicines [1]. For a long period of time, plants have been a valuable source of natural products for maintaining human health.

Morinda coreia belongs to Rubiaceae family wildly distributed throughout Southeast Asia. It is commercially known as Nunaa, Indian Mulberry, and small tree with immense medicinal properties. In traditional system of medicine, *Morinda coreia* leaves has been reported to have anti-convulsant, analgesic, anti- inflammatory, antioxidant and cytoprotective effect. Unripe fruit is used to cure rheumatism. Ash of the fruit prevents dysentery, vomiting, diarrhoea and cholera. There is greater demand for fruit extract of *Morinda* species in the treatment of arthritis, cancer, gastric ulcer and other heart disease [2].

There is no extensive activity reported on flowers of *Morinda coreia*. So the current study is undertaken to evaluate the pharmacognostical, phytochemical screening for dried powder and extracts of *Morinda coreia* flower. These pharmacognostical and phytochemical parameters will be useful for future investigators for identification of plant species.

II. MATERIALS AND METHODS

Plant collection

Fresh plant materials of *Morinda coreia* flowers were collected in morning from Amathur, Virudhunagar district, Tamilnadu, India during September month. The plant was identified and authenticated by botanist Dr.Stephen, senior lecturer, American college of arts &science, Madurai

Morphological evaluation

Morphological characters of crude *Morinda coreia* flower such as colour, shape, size, petals and corolla were assessed.

Microscopical evaluation

This study provides detailed information about the crude drug by virtue of its property to magnify the fine structures of minute objects to be visualized and thereby confirm the structural details of the plant drug under evaluation. It can also be used in the determination of the optical as well as micro chemical properties of the crude drug. In this study *Morinda coreia* flower was evaluated microscopically. The collected fresh flowers were immediately immersed in fixative fluid FAA (Formalin – 5ml + Acetic acid – 5ml +70% Ethyl alcohol – 90ml). After 24 hours of fixing, the specimens were dehydrated, filtered with paraffin wax. Jeffrey's maceration method was adapted to the small pieces of specimens. After maceration the tissues were washed in water and stained with toluidine blue. The stained tissues were mounted in glycerine and made observations with photomicrographs of different magnifications were taken with Nikon lab photo 2 Microscopic unit [3-5].

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Powder analysis of *Morinda coreia* flower

The flowers of *Morinda coreia* were shade dried, powdered and used for further validation such as powder microscopy, fluorescent and phytochemical analysis. The behaviour of powdered *Morinda coreia* flower with various chemical reagents [6]**.** The ash value, extractive value, loss on drying, swelling index and volatile oil content were evaluated [7-9].

Extraction

The powdered *Morinda coreia* flower was passed through sieve no 40. The coarse powder was stored in an air tight container. The extraction was carried out by cold maceration process, in which 250 gm of coarse powder was defatted with 500 ml of petroleum ether for 4 hours. Then it was filtered and the residue was air dried. To the air dried residue, 500 ml of ethanol, was added and kept aside for 72 hours with intermittent shaking. After 72 hours the product was filtered and the filtrate was concentrated and dried at room temperature using rotovapour. The same process was carried out to the marc left after filtration to get ethyl acetate and aqueous extracts.

Qualitative analysis

Primary and secondary metabolites were qualitatively screened by using the standard procedures [10, 11] for powder and extracts of *Morinda coreia* flower.

Quantitative analysis

The total phenol, flavonoid and tannin content were determined quantitatively in the extracts of *Morinda coreia* flower by using standard procedures with reference to standard marker compounds. [12-14]

Chromatography

Chromatography methods were important analytical tool in the separation, identification of different components present in crude plant extract [15, 16].

Thin layer chromatography (TLC)

The plates were prepared using silica gel-G and activated for separation. Based on the increasing polarity of mobile phase Toluene: Ethyl acetate: Formic acid: Methanol (3:6:1.6:0.4) was selected. The aqueous, ethanol and ethyl acetate extracts of *Morinda coreia* flower were dissolved in particular solvents to get a concentration of 100 mg/ml and 0.2µl of this solution was applied on the plate using capillary tube. Spots were detected under Visual and UV light at 254 and 366nm. The R_f values were calculated with reference to solvent and solute eluted.

High performance thin layer chromatography (HPTLC)

CAMAG Linomat 5 "Linomat5_170130" S/N 170130 (1.00.12) was used for the detection and Linomat 5 sample applicator was used for the application of the track. Twin trough plate development chamber was used for the development of chromatogram. Mobile phase Toluene: Ethyl acetate: Formic acid: Methanol (3:6:1.6:0.4) was used. The developed plates were examined at wavelength 200-400 nm. The R_f values and area under curve for extracts of *Morinda coreia* flower were examined [17].

III. RESULTS

The flowers are tubular, white, scented, about 2 cm long, corolla usually tomentosa outside, tube 1.3–2 cm long, anthers exseited. (Fig-1)

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Fig 1-Macroscopy of *Morinda coreia* flower

Morinda coreia flower powder was coarse in nature, greyish green colour, and characteristic odour with sour taste.

Many flowers are fused into a compound spherical body. The corolla tubes are few. The calyx is persistent on the surface of the fruit. The ground tissue is parenchymatous. The cells are wide, highly lobed and compact. There are numerous vascular strands distributed in the median part of the sepals. The sepals are 1.2mm thick. (Fig-2 A, B)

The powdered microscopy of *Morinda coreia* flower showed epidermal cells, large rectangular parenchyma cells with vascular strands raphides of calcium oxalate crystals. (Fig-2 C,D)

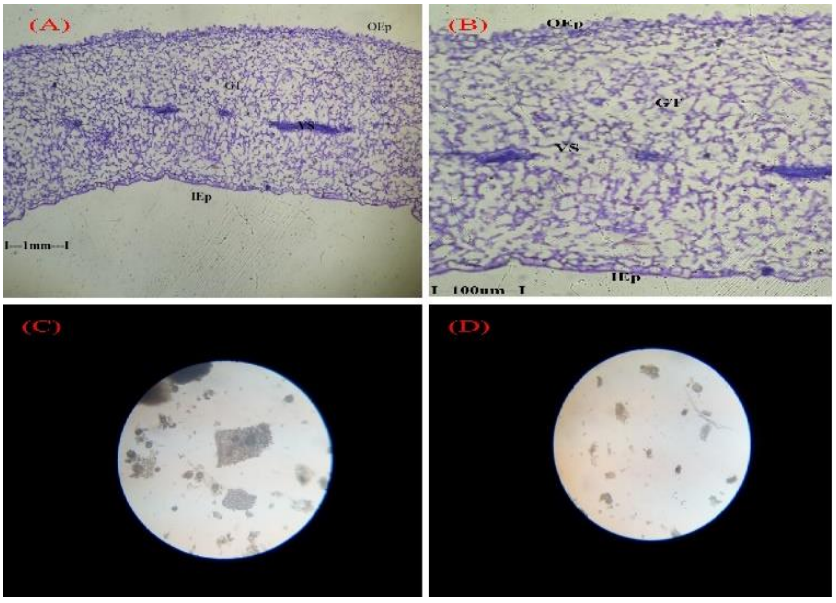

Fig 2-Microscopy of *Morinda coreia* flower

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The behaviour of *Morinda coreia* flower powder with various chemical reagents is presented in Table-1, which indicates the presence of phyto-constituents such as protein, phytosterols, tannins, starch, and flavonoids.

Table 1-Behaviour of *Morinda coreia* flower powder with various chemical reagents

Powder+ Reagents	**Colour/Precipitate**	**Presence of Active Principle**
Picric acid	Yellow precipitate	Presence of Protein
Conc.H_2SO_4	Reddish brown colour	Presence of Phyto sterols
Lieberman Burchard reagent	Reddish brown colour	Presence of Phyto sterols
$Fecl_3$	Greenish black colour	Presence of Tannins
Iodine solution	Blue colour	Presence of Starch
Mayer's reagent	No cream colour	Absence of alkaloid
Spot test	No stain	Absence of Fixed oil
Sulfosalicylic acid	White precipitate	Presence of Protein
Aq.NaOH	Yellow colour	Presence of Flavonoids
Mg-Hcl	Magenta colour	Presence of Flavonoids
Aq.Lead acetate	White precipitate	Presence of Tannins

The organic molecules absorb light usually over a specific range of wave length and many of them remit such radiations. So if the powder is treated with different chemical reagents and seen in the UV chamber, different colours were produced.

These parameters are useful for quality control and purity checking of the plant in powder form and its crude extracts. The results of the fluorescence analysis of *Morinda coreia* flower powder and its extracts are tabulated in the Table-2, 3.

Powder +Reagent	**Day light**	**Short UV**	**Long UV**
Drug powder	Greyish brown	Brown	Dark Brown
Drug powder + Aq 1M NaoH	Brown	Yellow	Green
Drug powder + Alc.1M NaoH	Brown	Yellow	Green
Drug powder + glacial acetic acid	Brown	Light green	Brownish green
Drug powder + 50% Hcl	Brownish green	Yellowish green	Dark Brown
Drug powder + 50%H_2SO_4	Green	Light green	Dark green
Drug powder + 50%HNO_3	Light green	Light green	Green

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Drug powder + iodine	Red	Brown	Brown
Drug powder + 10% KOH	Yellow	Brownish Yellow	Green
Drug powder + 1M Hcl	Brownish green	Yellowish green	Dark Brown

Table 2-Fluorescence analysis of *Morinda coreia* flower powder

Table 3-Fluorescence analysis of various extracts of *Morinda coreia* flower

Extracts	Consistency	Colour in Visible Light	Colour in Short UV	Colour in Long UV
Aqueous	Semisolid	Dark Brown	Brown	Yellow
Benzene	Semisolid	Brownish Green	Brown	Yellow
Chloroform	Semisolid	Greenish Brown	Green	Yellowish Green
Ethanol	Semisolid	Greenish Yellow	Brown	Green
Ethyl Acetate	Semisolid	Dark Green	Brown	Green
Methanol	Semisolid	Greenish Brown	Orange	Brown
Pet Ether	Semisolid	Greenish Brown	Greeen	Brownish Black

The various Physio-chemical parameters such as moisture content, ash values, foreign organic matter, extractive values, foaming index, swelling index and volatile oil content were determined for *Morinda coreia* flower and its powder. The results obtained for Physio-chemical parameters were presented in Table-4.

Table 4-Physio-Chemical Parameters of *Morinda coreia* Flower

Parameters	Values Expressed As %
Moisture Content	2.076±0.0023
Foreign Organic Matter	0.06±0.073
Ash Values	
Total Ash	8±0.016
Acid Insoluble	5.7±0.004
Water Soluble	1.07±0.002
Sulphated Ash	1.1±0.052
Extractive Values	
Aqueous	23.6±0.052
Benzene	3.8±0.001
Chloroform	18.6±0.075
Ethyl Acetate	26.4±0.064

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Ethanol	28.8±0.041
Methanol	9±0.1.8
Pet Ether	20.6±0.009
Foaming Index	Less Than 100
Swelling Index	Expressed As Ml
Initial Volume	2.7±0.14
Final Volume	4.9±0.152
Volatile Oil	0.43±0.0023

*mean of three readings ± SEM

The determination of ash values helps to find out whether the powdered material was adulterated with sand or any other inorganic material. The extractive values help us to decide which solvent will be useful for extraction of maximum active principles and also helps to decide whether the crude material has been exhausted or not.

The physiochemical parameters of *Morinda coreia* flower gave the valuable information regarding the morphology of crude drugs. It can be useful for the authentication of this plant among all species of *Morinda* and to judge the adulteration and purity of this drug. Since the parameters are constant and any change in these values are indicative of substitution and adulteration of the plant materials.

The *Morinda coreia* flower powder was extracted by maceration process. The quantity of the extracts obtained by using various solvents (Ethanol, Ethyl acetate, Aqueous) and its nature are tabulated in the Table-5.

Table 5 – Nature of the extracts

Extracts	**Colour**	**Consistency**	**Quantity(gms)**
Ethanol	Greenish Yellow	Semisolid	3.38
Ethyl acetate	Dark Green	Semisolid	3.71
Aqueous	Dark Brown	Semisolid	4.25

From the qualitative phytochemical screening study it has been estimated that the powder and various extracts of *Morinda coreia* flower contains all the necessary primary and secondary metabolites such as carbohydrates, proteins sterols, tannins, saponin, triterpenoids and flavonoid, except alkaloids and glycosides ,the results were tabulated in Table-6.

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Table 6-Phytochemical screening for powder and various extracts of *Morinda coreia*

Test	Powder	Pet.Ether	Chloroform	Ethyl acetate	Ethanol	Aqueous
Test for sterols						
a.Salkowski's test	+	+	+	+	+	-
b.Libermann-burchard's test	+	+	+	+	+	-
Test for carbohydrates						
a. Molisch's test	+	-	-	+	+	+
b. Fehling's test	+	+	+	+	+	+
c. Benedict's test	+	+	-	+	+	+
Test for Proteins						
a. Million's test	+	-	-	-	-	-
b. Biuret test	+	-	-	-	-	-
Test for alkaloids						
a.Mayer's reagent	-	-	-	-	-	-
b.Dragendorff's reagent	-	-	-	-	-	-
c.Hager's reagent	-	-	-	-	-	-
d.Wagner's reagent	-	-	-	-	-	-
e.Test for Purine group (Murexide test)	-	-	-	-	-	-
Test for glycosides						
a.Anthraquinone glycosides						
i)Borntrager's test	-	-	-	-	-	-
ii)Modified Borntrager's test	-	-	-	-	-	-
b.Cardiac glycosides						
i)Keller-Killiani test	-	-	-	-	-	-
c.Cyanogenetic glycosides	-	-	-	-	-	-
Test for saponin	+	-	-	+	+	+
Test for tannins						
Fecl3 test	+	+	+	+	+	+
Test for flavonoids						
a. Shinoda test	+	-	-	+	+	-
b. Alkali test	+	-	-	+	+	+
c. Acid test	+	-	-	+	+	-
d.Ammonia test	+	-	-	+	+	+
Test for terpenoid	+	+	+	+	+	-
Test for mucilage	+	-	-	+	+	-
Test for volatile oil	+	-	-	-	-	-

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In quantitative estimation, a particular group of compound present in the crude ethanolic extract were quantified by means of using standard marker compound and reporting them as equivalent to that much amount of compound present in the extract as per standard compound. In this study the total phenolic, flavonoid and tannin content were estimated.

The total phenolic content was estimated by using folin-ciocalteu reagent. The amount of phenolic content was found in terms mg GAE/gm. The total flavonoid content was determined by using aluminium chloride. The amount of total flavonoid content was found in terms mg rutin/gm of the extract. The total tannin content of extracts was determined by using Folin-Denis reagent. The amount of tannin content was found in terms of mg tannic acid/gm. The results for the total phenol, flavonoid and tannin contents were tabulated in Table 7.

Table 7. Total flavonoid, tannin, phenol content

Extracts	Flavonoid	Tannin	Phenol
Aqueous	52.00±0.17	86.50±0.39	67.53±0.67
Ethanol	65.33±0.19	94.00±0.30	83.24±0.48
Ethyl Acetate	62.33±0.24	52.00±0.37	66.46±0.55

*mean of three readings ± SEM

The number of spots obtained in extracts of *Morinda coreia* flower by TLC analysis with mobile phase toluene: ethyl acetate: formic acid: methanol (3: 6: 1.6: 0.4) were observed**.** The extracts showed many spots at visible light, short UV (254 nm) and long UV (365 nm). The pictorial image was quoted in Fig-3. The R_f values were calculated and represented in the Table-8 to 10.

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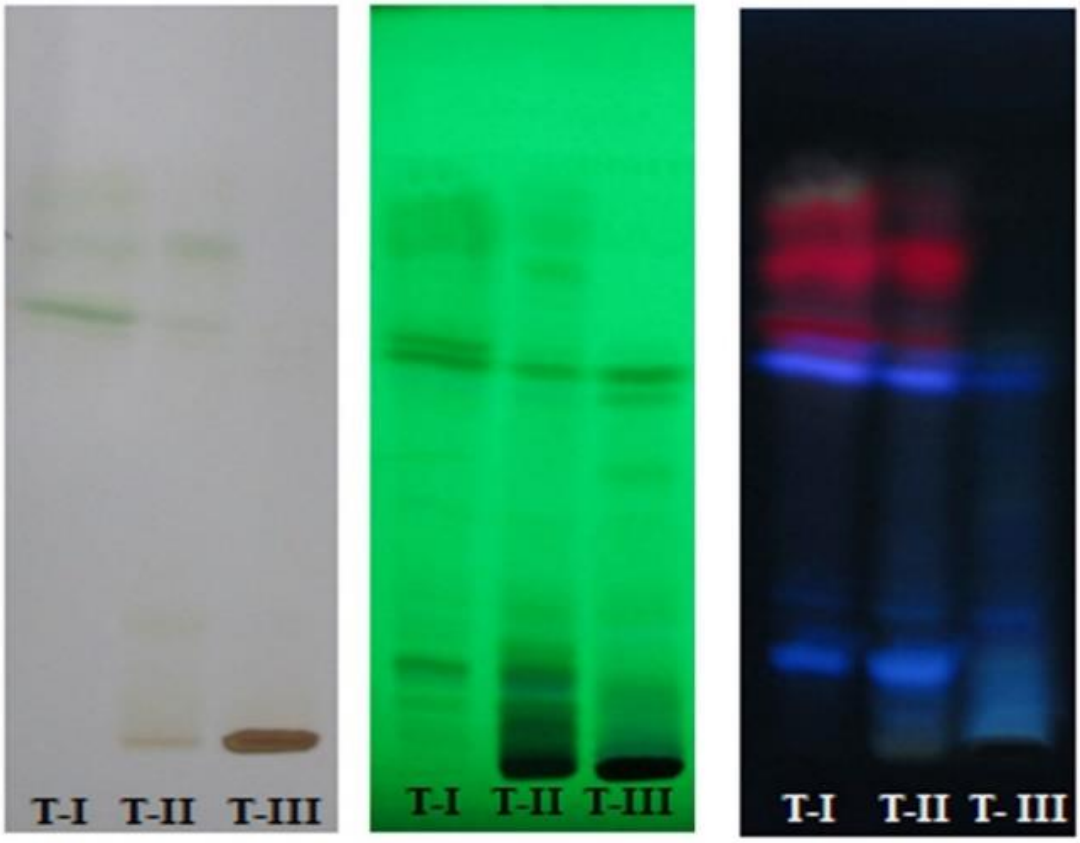

Figure 3. Spots under UV light

T-I Ethyl acetate extract of *Morinda coreia*
T-II Ethanolic extract of *Morinda coreia*,
T-III Aqueous extract of *Morinda coreia*

Table 8-Colour of the spots under visible light

Name of the extract	Solvent system	No of spots	Colour of the spot under Visible light	R_f value
Ethyl acetate	Toluene : Ethyl acetate: Formic acid: Methanol (3:6:1.6:0.4)	1	Light Green	0.69
		2	Light Green	0.72
		3	Light Green	0.83
		4	Light Green	0.98
Ethanol		1	Light Brown	0.07
		2	Light Brown	0.11
		3	Light Brown	0.17
		4	Light Green	0.65
		5	Light Green	0.76
Aqueous		1	Light brown	0.15
		2	Light brown	0.59

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Table 9-Colour of the spots under short UV at 254 nm

Name of the extract	Solvent system	No of spots	Colour of the spot under UV at 254 nm	R_f value
Ethyl acetate	Toluene: Ethyl acetate: Formic acid : Methanol (3:6:1.6:0.4)	1 2 3 4	Light Green Light Green Light Green Light Green	0.1 0.17 0.65 0.92
Ethanol		1 2 3 4 5	Light Green Light Green Light Green Light Green Light Green	0.07 0.1 0.17 0.53 0.92
Aqueous		1 2 3	Greenish brown Greenish brown Greenish brown	0.12 0.59 0.65

Table 10-Colour of the spots under Long UV at 365 nm

Name of the extract	Solvent system	No of spots	Colour of the spot under UV at 365 nm	R_f value
Ethyl acetate	Toluene: Ethyl acetate :Formic acid: Methanol (3:6:1.6:0.4)	1 2 3 4 5 6 7	Blue Fluorescence Violet Fluorescence Blue Fluorescence Pink Fluorescence Orange red fluorescence Orange red fluorescence Orange red fluorescence	0.13 0.23 0.6 0.63 0.67 0.72 0.86
Ethanol		1 2 3 4 5 6 7 8	Blue Fluorescence Blue Fluorescence Blue Fluorescence Blue Fluorescence Blue Fluorescence Orange red Fluorescence Orange red Fluorescence Orange red Fluorescence	0.05 0.13 0.22 0.6 0.53 0.65 0.79 0.82
Aqueous		1 2 3	Blue Fluorescence Blue Fluorescence Blue Fluorescence	0.09 0.21 0.53

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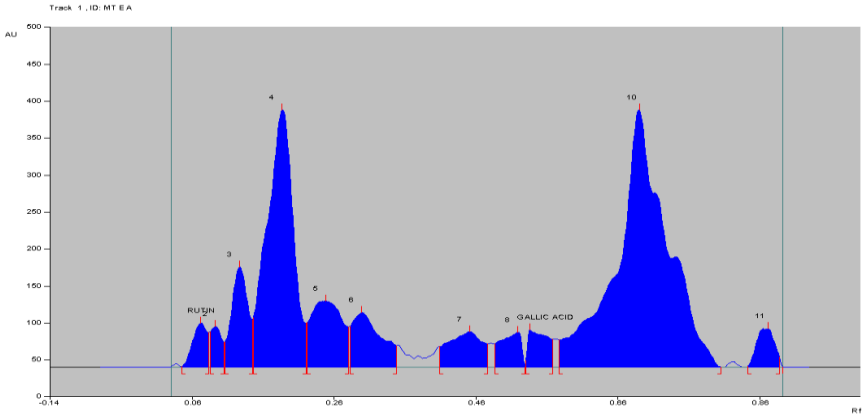
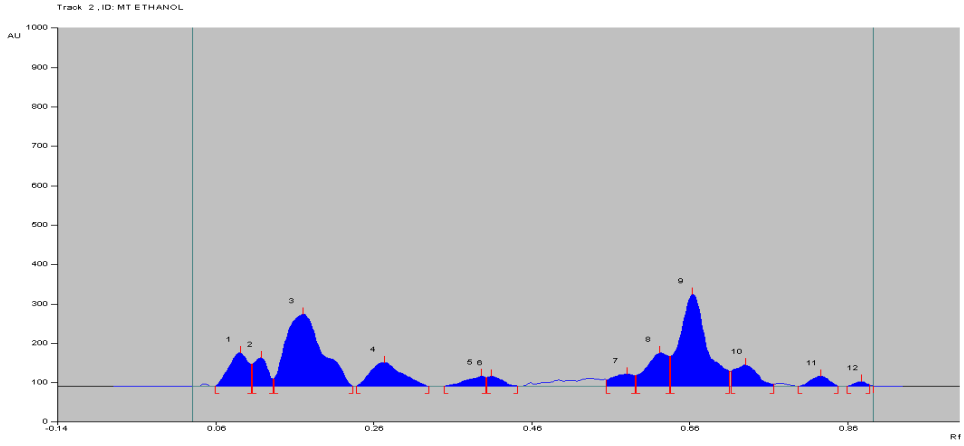
From HPTLC analysis, the R_f value of EAEMC (peak 1, 9) coincides with standard flavonoid (Rutin) and tannin (Gallic acid) with R_f value 0.07, and 0.53 respectively. From these findings, it reveals that the phytoconstituents present in EAEMC was assigned as Rutin (0.07) and Gallic acid (0.53). The chromatogram was presented in Fig-4.

Fig 4-Chromatogram of Ethyl acetate extract of *Morinda coreia*



The R_f value of EEMC (peak 9) coincides with standard flavonoid (Quercetin) R_f value 0.65. From these findings reveals that the phytoconstituents present in EEMC was assigned as Quercetin (0.65). The chromatogram was presented inFig-5.

Fig.5-Chromatogram of Ethanolic extract of *Morinda coreia*



The R_f value of AEMC (peak 7) coincides with standard flavonoid (Quercetin) with R_f value 0.65. From these findings it reveals that the phytoconstituents present in AEMC was assigned as Quercetin (0.65). The chromatogram was presented in Fig-6

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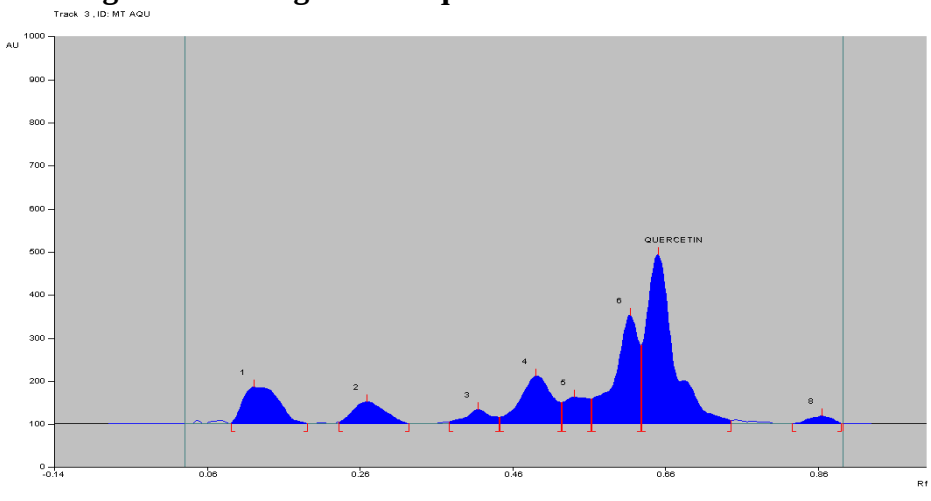
Fig.6-Chromatogram of Aqueous extract of *Morinda coreia*



IV. DISCUSSION

The pharmacognostical evaluation describes the morphology, microscopy and physio-chemical parameters of *Morinda coreia* flower. These findings play an important role in the authentication of crude drug and will be useful for the determination of quality and recognise the plant for feature work.

The phytochemical evaluation deals with the preliminary phytochemical screening and quantitative estimation of phytoconstituents present in powder and different extracts of *Morinda coreia*, which confirms the presence of secondary metabolites like flavonoids, saponins, sterols, and tannins but alkaloids and glycosides were absent in *Morinda coreia* flower powder and extracts.

The preparation of *Morinda coreia* flower extract was carried out by maceration process. The amount of total phenols, total tannins and total flavonoid content in extracts of *Morinda coreia* flower were estimated. This determination confirms the major concentration of the phytoconstituents in the EEMC.

Chromatographic separation of therapeutically important phytoconstituents in different extracts of *Morinda coreia* flower (EEMC, EAEMC and AEMC) was carried out by TLC and HPTLC. HPTLC provides a chromatographic fingerprint of phytochemicals and is suitable for confirming the identity and purity of medicinal plant raw material. The presence of therapeutically active phytoconstituents was found to be Quercetin, Rutin and Gallic acid in the extracts of *Morinda coreia* flower by comparing with the standard R_f values.

V. CONCLUSION

WHO has emphasised the need to ensure quality control of the raw materials used for herbal medicines by using modern techniques and by applying suitable parameters and standards. In the present investigation various standardization parameters such as macroscopy, microscopy, physico-chemical constants, preliminary phytochemical investigation,

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HPTLC profiles were studied, which are being reported for the first time in this part of the plant and could be helpful in authentication and preparation of a suitable monograph for the proper identification of *Morinda coreia* flower.

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