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In-Vitro Antibacterial Activity, Phytochemical Screening and Characterization of Morinda Tinctoria Root Extracts

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ABSTRACT

The objective of the present investigate to evaluate the presence of phytochemical constituents, characterization and antibacterial activity of different extracts of roots of Morinda Tinctoria. The extraction was done with the series of solvents like hexane, ethyl acetate and methanol with increasing polarity using soxhlet apparatus. The phytochemical analysis was done by using the slandered procedure. The Plant extracts were characterized by UV-vis, FT-IR spectrum. These extracts were tested for antibacterial activity against four human pathogens viz., Streptococcus aureus, Bacillus subtilis, Pseudomonas Auregenosa, Escherichia coli. The results revealed that the roots extracts contain phytochemical constituents like Alkaloids, Phytosterols, Flavonoids, Phenols and Triterpenes. Ethyl acetate extract shows more effective against the gram-positive organisms as well as gram-negative organism, followed by hexane and methanol extracts. The UV-vis spectrum profile showed different peaks ranging from 232nm to 372nm with different absorption respectively. The presence of active compounds was identified by FT-IR. The present study concludes that the different extract of M.Tinctoria roots contains good antibacterial activity against tested microorganisms. The present study concluded that M. Tinctoria roots extracts can be investigated to discover the bioactive natural products that may present as leads in the improvement of new pharmaceuticals.

Keywords: Morinda Tinctoria roots, UV-vis, FT-IR, and antibacterial activity.

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INTRODUCTION

Morinda Tinctoria, commonly known as Indian Mulberry (Rubiaceae) native in South Asia, Eastern India and Pacific islands. The species Morinda grows wildly grows and distributed in South India. Many species of M. Tinctoria are presented in India. The M.Tinctoria mostly grows a like wild plant tree in vacant agricultural land. Morinda has been long cultivated in different parts of Tamil Nadu state in India. Although the south Indian associates realized the therapeutic value of M.Tinctoria and used it in the traditional Indian medicinal systems like Siddha [1-13].Oxidative stress is now be linked with more than 100 diseases, as well as with a normal period diseases like Stoke, Diabetes, Cancer, Cardiovascular diseases, AIDS and neurogenerative diseases, etc. [14]. Oxidative stress has also been predictable to be involved in the etiology of liver diseases [15]. The most common diseases like cancer and the third most common cause of cancer-related death [16]. Plants have a long history in the treatment of different cancer cells [6].Natural antioxidant have been proposed and utilized as therapeutic agents to counteract liver damage [17]. Many species of Morinda genus have been reported for various health disorders and anticancer activity by Indian pharmacopoeia. For instance, M.Tinctoria, which is also called Noni or Yor, contain several medicinally active components that exhibited various therapeutic effects. These include antibacterial, antiviral, and anticancer activities as well as analgesic effects [18, 19].It is evergreen shrub growing to 5-10 m tall. Long stipules membranous broad entire the variable in size. It is evident from the earlier literature that it can be used as astringent, deobstrent, emmenagogues and to relieve pain in the gout [20].The green fruit and leaves are used to treat menstrual cramps, bowel irregularities and urinary tract infections. Hence to explore the best solvent for the phytochemical screening the said plant is selected for pharmacological studies [21]. In this present study the extraction, screening, characterization and biological activity of the M.Tinctoria roots has been carried out.

Taxonomy and Nomenclature (Common Names) Is As Following

Kingdom: Plantae,
Order: Gentianales,
Family: Rubiaceae,
Genus: Morinda,
Species: M. Tinctoria.,

It is known by the vernacular languages as given in table 1.

S.No	Language	Name
1	Tamil	Nuna Tunava,
2	Telugu	Maddi chettu
3	Hindi	Al Achi,
4	Malayalam	Pavitto,
5	Sanskrit	Achuka
6	Kannada	Haladipavete,
7	English	Indian Mulberry

MATERIALS AND METHODS

Plant Collection

M. Tinctoria plant was identified and collected from Sathoor, Viruthunagar District Tamil Nadu. The fresh plant material was air dried as shown in Fig.1 A and then grained to get homogenize fine powder as shown Fig.1 B.

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Fig. 1: A. small size of the root material **B. Powder of the root material**

Extraction of Morinda Tinctoria Roots

The plant material was extracted by using Soxhlet apparatus at 50-60^{0}C as shown in fig.2. In the extraction procedure, the total amount of 80 gms powered was used. The extraction was carried out by using increasing polarity starting from the extract of n-Hexane, ethyl acetate and methanol respectively.

Fig.2. Soxhlet apparatus for the M.Tintoria roots extraction

The extraction was carried out with 800 ml of each solvent for the period of 6-8 hours. At the end of the extraction, the respective solvents were concentrated by using the rotary evaporator at 40-50^{0}C under reduced pressure as shown in fig.3. The following extracts of M.Tinctoria were obtained and named as

HEMT – Hexane extract of the root of Morinda Tinctoria
EAMT- Ethyl acetate extract of the root of Morinda Tinctoria,
MEMT - Methanol extract of the root of Morinda Tinctoria

Fig.3 Rotary Evaporator for Concentration

Phytochemical Screening

Phytochemical screening of M.Tinctoria roots extract was carried out according to the methods described by Harborne [22], Trease and Evans [23] and Sofowara [24].

UV-Vis Spectroscopy

The extracts of M.Tinctoria roots were monitored by UV-Vis spectrum of the reaction mixture after diluting a small aliquot of the sample with DMSO. The optical absorbance between 200 and 1100 nm with a UV-Visible spectrophotometer (Elico BL 180 Bio) was recorded.

FT-IR (Fourier Transform Infra Red) Spectroscopy

A single-beam FT-IR spectrometer was used. The FT- IR spectrum was recorded using KBr disc for the successive extracts.

In-Vitro Antibacterial Activity.

The extracts was screened In-vitro studies for their antibacterial activity against both gram positive and gram negative bacterial strains by sop for Disc Diffusion method using cultivated on a suitable agar medium under optimal incubation conditions to obtain a fresh overnight grown culture Bauer et-al 1966 [25].The bacterial strains used for the determination of antibacterial activity are Escherichia coli, Staphylococcus aureus, and Bacillus subtilis Pseudomonas aeruginosa.

DAY 1

1. The solution of the extracts was prepared at 5ml in dimethyl sulfoxide (DMSO).

DAY 2

2. Harvest a number of distinct colonies from the fresh grown plate culture to suspend in a tube containing broth until turbidity visually corresponding to 1.0 McFarland standard is reached. Using a sterile cotton swab dipped into the adjusted culture medium and squeezed. Then made a lawn culture on Muller – Hinton Agar media. Allow to dry the plates for max. 15 minutes. Longer drying times allow pre-incubation of the cells that should be avoided. Plates should be incubated as soon as possible after the application of the discs. Using sterile forces, the discs Antibiotic or tested compound loaded) are applied onto the agar surface. Discs must not be relocated once they have made contact with the agar surface. Incubate the plates under optimal incubation conditions.

DAY 3

3. The diameter of the inhibition zones are measured to the nearest mm from the point of abrupt inhibition of growth (using a calipers or mm ruler).

RESULTS AND DISCUSSION

Phytochemical screening evaluation of the various extracts of the roots of M.Tinctoria was studied for the presence of Alkaloids, Saponins, Phytosterols, Carbohydrates, Proteins, Amino acids, Tannins, and Flavonoids, and the results are presented in Table 2.

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Table 2: Phytochemical screening of roots extracts of M. Tinctoria

S.No	Test	HEMT	EAMT	MEMT
1.	Test for Alkaloids			
	Dragendraffs test	+	+	+
	Wagner test	+	-	-
	Hagers test	+	+	+
2.	Test for Flavonoids			
	Lead acetate test	-	+	+
	NaOH	-	+	+
3	Test for Phenols			
	$Fecl_3$	-	-	-
4.	Test for Tannins			
	$Fecl_3$	+	-	+
	$K_2Cr_2O_7$	-	-	+
	Lead acetate	+	+	+
5.	Test for saponins			
	Form test	+	+	-
6.	Test for Amino Acid			
	Xantho protic test	-	+	+
	Biuret Test	+	-	-
7.	Test for Coumarin	-	-	-
8.	Test for Starch (Iodine test)	-	-	-
9.	Test for Quinone	-	+	+
10.	Test for Carbohydrates			
	Fehling test	-	+	-
	Benedict test	-	+	+
	Molishs test	-	-	-
11.	Test for cardiac glycosides			
	Killer –Killani test	-	-	-
12.	Test for terpenoids			
	Salkovaski test	+	-	-
	Lieberman test	-	-	-
13.	Test for phytosterol			
	Salkovaski test	+	-	-
	Lieberman test	+	-	-
14.	Test for Anthraquinone			
	Extraction $+NH_4OH$	+	-	-
	Benzene Test	+	-	+

+ Present, - Absent

The test data available in Table .2 indicated that all the three extracts of M.Tinctoria roots contained alkaloids, flavonoids, tannins, saponins, amino acid, terpenoids, phytosterol and anthraquinone. It is evidently

from the table that the other phytoconsituents like phenols, coumarins and glycosides were absent in all the three solvent extracts. These results suggest the presence of primary bioactive metabolites that acts as the precursors for the synthesis of secondary metabolites. These turns help in the development of new bio products for future.

UV-Vis Spectrum

The qualitative UV-vis spectrum of M.Tinctoria of solvents extract (N-Hexane, Ethyl acetate and Methanol) was selected at were wavelength 200nm to 1100nm.

Table 3: UV-Visible spectrum peak values of different solvent extracts of Morinda Tinctoria

S.NO	Solvent	Wavelength (nm)	Absorption Value
1	n-Hexane	232	1.0496
		312	4.01619
		362	4.0358
2.	Ethyl acetate	272	1.10999
		282	2.1666
		372	3.6916
3.	Methanol	247	1.24499
		322	4.0832

The comparative UV-Vis spectra of n-Hexane, ethyl acetate and methanol extract were records as ethanol being considered as a reference solvent. The UV-Vis spectrum of crude extracts of absorption maximum (Wavelength) given in table -3. It has been observed that all the three different solvents extracts like flavonoides derivatives but n-hexane is alkaloids derivatives. The UV-Vis spectrum of M.Tinctoria roots extracts was shown in Fig. 4, 5 and 6 respectively as shown below

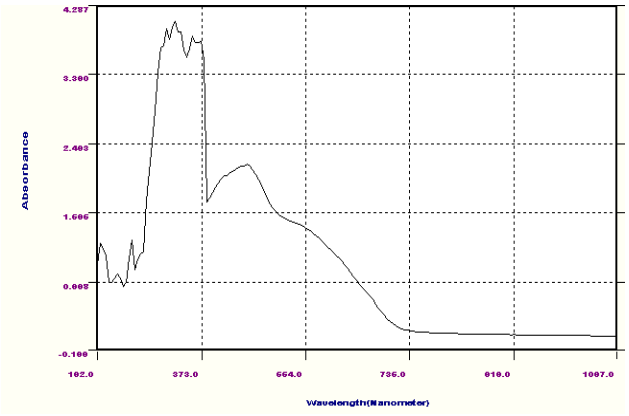

Fig. 4: UV-Vis Spectra of M. Tinctoria roots extract of Methanol

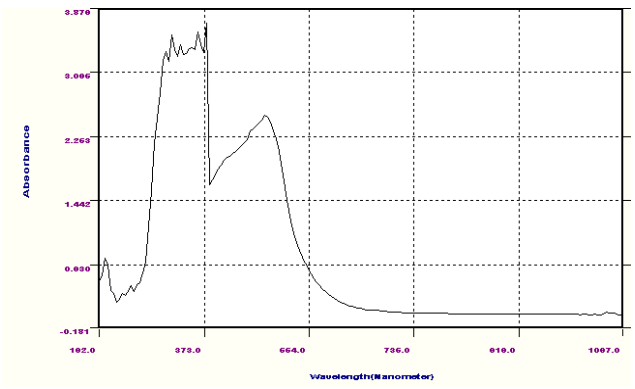

Fig .5: UV-Vis Spectra of M. Tinctoria roots extract of Ethyl acetate

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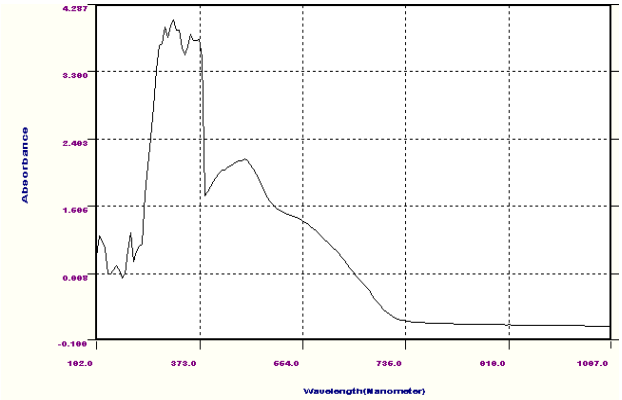

Fig.6: UV-Vis Spectra of M.Tinctoria roots extract of Hexane

FT-IR – Spectrum

The spectrum was recorded in the wavelength region between 400cm^{-1} to 4000cm^{-1}. The spectrum shows peaks at 3422cm^{-1}, 3420cm^{-1}, which indicates the presence of –O-H stretching of the carboxyl group and N-H stretching of secondary amides. Further, the peaks observed at 2922cm^{-1}, 2924cm^{-1} represents the C- H stretching bonds of alkanes. The peak observed at 1260cm^{-1}, 1599cm^{-1}, 1364cm^{-1}, 1588cm^{-1}, 1348cm^{-1}, 1593cm^{-1}, represent the C-O alcohols, carboxylic acids etc. The sharp peak at 1046cm^{-1} is assigned to aliphatic amine. The peak observed at 712cm^{-1},801cm$^{-1,}$ and 722 cm^{-1} represent the presence different functional groups like phenyl ring substitution bands C-H bending.

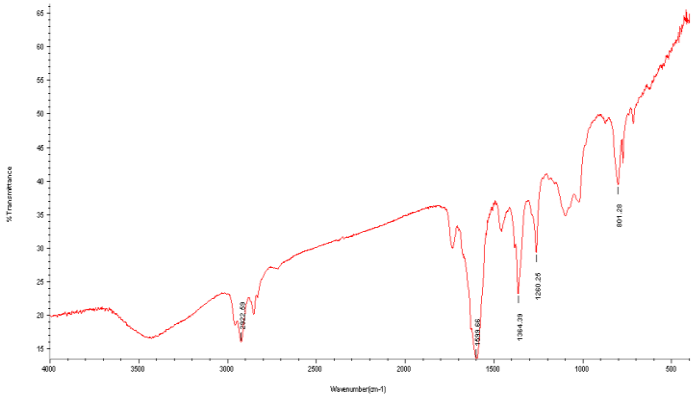

Fig. 7: FT-IR Spectrum of M. Tinctoria roots extract of n-Hexane

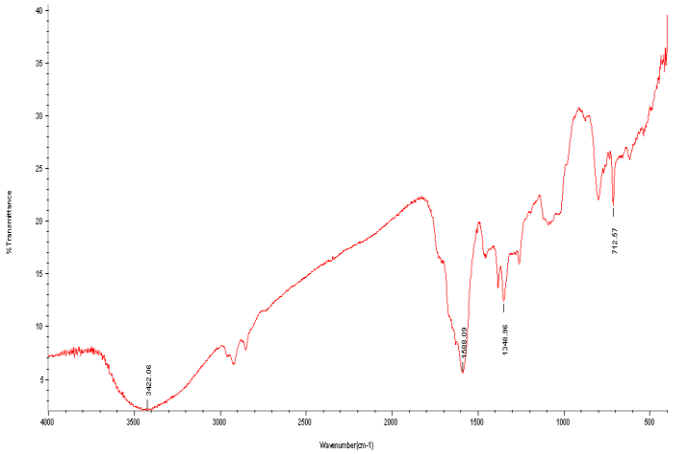

Fig. 8: FT-IR Spectrum of M. Tinctoria roots extract of ethyl acetate

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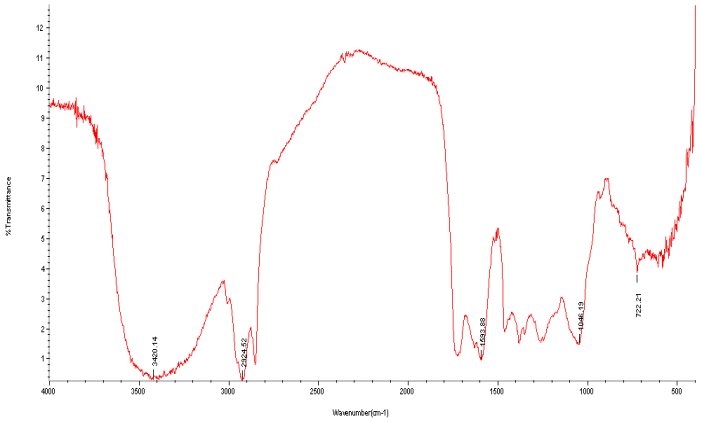


Fig. 9: FT-IR Spectrum of M. Tinctoria roots extract of methanol

In-Vitro Antibacterial Activity

Antibacterial activity of M.Tinctoria roots extracts was carried out on four human pathogens, such as Streptococcus aureus, Bacillus subtilis are gram +ve and Pseudomonas Auregenosa and Escherichia coli are gram -ve bacteria [Fig-10]. The results for anti- bacterial activity of M.Tinctoria roots extracts showed clear zone of inhibition as indicated in Table-3 against Streptococcus aureus, Bacillus subtilis, Pseudomonas Auregenosa, Escherichia coli. The concentration of 50µg/ml was used as +ve as well as –ve control. The crude M.Tinctroia may show antibacterial activity against B.Subtilis for higher antibacterial activity

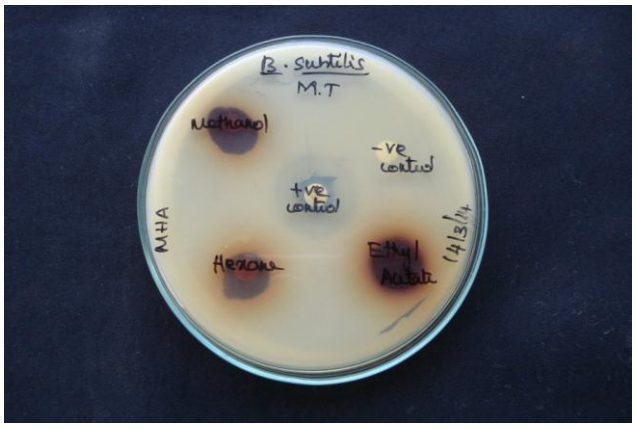
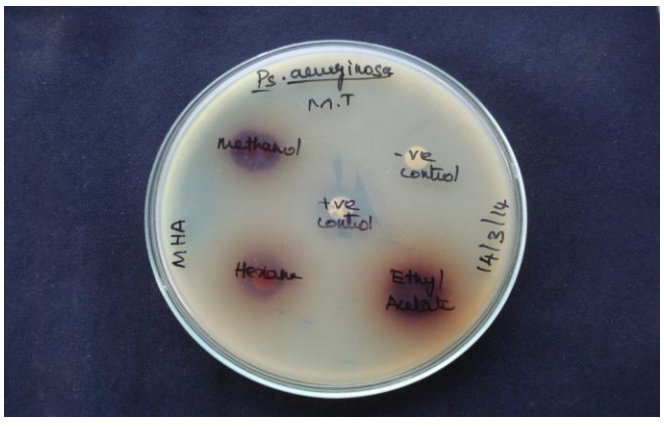


Figure.10: Zone of inhibition of M.Tinctoria roots extracts using different solvents against Bacterial Pathogens. A) B.Subtillis



B) P.aeruginosa

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Table 4: Antibacterial activity of various roots extracts of M.Tinctoria (Zone of inhibition in mm)

S.No	Extracts	Concentration	Diameter of zone of inhibition (mm)			
			G+ve		G-ve	
			S.aureus	B.subtilis	P.aeruginosa	E.coli
1.	HEMT	50µg/ml	NA	6.5mm	5.5mm	NA
2.	EAMT	50µg/ml	NA	5.5mm	5.5mm	NA
3.	MEMT	50µg/ml	NA	6mm	5.5mm	NA

The gram +ve bacterial strain using M.Tinctoria roots of hexane extract were shown to be as potent inhibition zone of is 6.5 mm ethyl acetate shown to be zone of inhibition, 5.5 mm followed by methanol shown to be zone of inhibition, 6.0 mm. The gram –ve bacterial strains was shown the same zone of inhibition (5.5mm). The order of the antibacterial activities is Hexane > Methanol > Ethyl acetate extract. The results clearly show that alkaloids. Phytosterols, Flavonoids, tannins and terpenes which were abundantly found in Methanol, Ethyl acetate and Hexane extracts were responsible for the antibacterial activity of M.Tinctoria roots. The antibacterial studies are given in table 4 and shown in figure.10 revealed the significant antibacterial potential of all the three extracts of M.Tinctoria roots. The antibacterial activity of the extracts might be attributed to the presence of the secondary metabolites in the extracts.

CONCLUSION

The antibacterial potential of the n-hexane, ethyl acetate and methanol extracts of the M.Tinctoria roots shows the inhibition on Bacillus subtilis, Pseudomonas Auregenosa. It could be concluded that M.Tinctoria roots extracts contain a number of pharmaceutically important photochemical constituents like alkaloids, saponin. Phytosterols, terpenes, carbohydrates, tannins, flavonoids and amino acid. The maximum zone of inhibition (6.5 mm) was obtained against Bacillus subtilis at a concentration of 50µg/ml. Extracts containing n-Hexane and Methanol shows more efficient in the antibacterial activity than the other extracts. UV–Vis spectra of the extracts shows that the sharp bands of extracts were observed at 232 nm to 372 nm absorption spectra of hexane, ethyl acetate and methanol indicated that the alkaloids and flavonoids derivatives. The FT-IR studies confirmed the presence of the carbonyl group for the amino acid and aromatic compounds in the plant extracts.

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