Journal of Science and Technology
ISSN: 2456-5660 Volume 7, Special Issue 03 (MAY 2022)

www.jst.org.in *DOI:https://doi.org/10.46243/jst.2022.v7.i03.pp25-31*

Phytochemical, Antibacterial, Metal Content and Spectral Studies of *Morinda Tinctoria*

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To Cite this Article
Pramila Ghumare, Dattatraya Jirekar "**Phytochemical, Antibacterial, Metal Content and Spectral Studies of *Morinda Tinctoria***", *Journal of Science and Technology, Vol. 07, Special Issue 03, May 2022.*

Article Info
Received: 19-04-2022 *Revised: 9-05-2022* *Accepted: 11-05-2022* *Published: 21-05-2022*

Abstract:*Plants have been one of the important sources of medicines, since the beginning of human civilization. Plant based medicines, food supplements, health products, cosmetics and pharmaceuticals are in the great demand these days. Nuni is the commercial name for Morinda tinctoria (L), which belongs to the family Rubiaceae. It is used in the treatment of various diseases. Different parts of Morinda tinctoria (L) such as roots, fruits and leaves are used as an astringent, deobstruent in the treatment of illness such as cancer, gout, arthritis, heart diseases and gastric ulcer, etc. The ashes of Morinda tinctoria (L) leaves act as biosorbents which controls ammonia pollution in waste waters. This study was carried out to evaluate the phytochemical and potential antimicrobial activity against five bacterial strains namely Staphylococcus aureus, Salmonella typhimurium, Proteus vulgaris, Pseudomonas aeruginosa and B. megaterium by agar cup assay method. The acetone leaf extracts of Morinda tinctoria was proved to be highly antibacterial activity as compared to other extract. Due to rich source of primary and secondary metabolites Morinda tinctoria exhibiting the antimicrobial activity. Morinda tinctoria evaluated for nickel, copper, cobalt and iron content. It shows 20.0 ppm of nickel, 82.6 ppm of copper, 4.1 ppm of cobalt and 8154.9 ppm of iron. Also, UV-Visible and FTIR of this different extract has been done.*

Key Word*:Morinda tinctoria, Phytochemical analysis, Antimicrobial Activity, Metal content, Spectral study.*

Introduction

India is known for its rich diversity of medicinal plants and from ancient times these plants have been utilized as therapeutic agents [1]. Many bioactive compounds and medicinal power are possessed by the medicinal plants. Bioactive compounds and medicinal power have great pharmacological significance it depends on phytochemical constituents. 119 secondary metabolites were identified by the researchers that are isolated from the plants being used as drugs globally. More than 80% of the world's population has been using the traditional medicines as primary health care needs [2]. Different valuable chemical components like phthalides, terpenoids, aromatic compounds, alkynes, alkaloids, sterols, fatty acids, tannins, anthocyanin, phenylpropanoids, essential oils, polysaccharides and phenolic compounds etc are included in natural products. They also have significant antioxidant activity [3]. Apart from their role of health benefactors, antioxidants are added in foods to prevent or delay oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature [4].

Medicinal value has been found in thousands of species in India. Since ancient times it has been a practice to use several medicinal plants and its different parts to cure specific diseases [5]. Being valuable natural resources medicinal plants are considered safe drugs. These drugs have been tested for antimicrobial, biological, as well as hypoglycemic activity. These activities play a vital role in the world of modern medicine [6,7]. It is well known that even the most synthetic drugs have their origin from plant products [8]. There are two reasons behind rapid

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increase in scientific attraction towards medicinal plants. The first being the growing concern in plant derived drugs and the second reason is the raising interest in the side effects of modern medicine.

Morinda tinctoria belonging to the family Rubiaceae is a multipurpose tree that grows wild and is distributed throughout South East Asia. It is commercially known as Nuni and is indigenous to tropical countries. *M. tinctoria* is considered to be an important folklore medicine. Pain in the gout is relieved with the leaves of M. *tinctoria* and it works as an astringent, deobstruent [9]. There is a greater demand for the fruit extracts of Morinda species for the treatment of different kinds of illness `such as arthritis, cancer, gastric ulcer, heart diseases etc [10]. Ammonia pollution in waste water is also controlled by ashes of *Morinda tinctoria* leaves and it acts as bio sorbents [11].

Toxonomical classification of *Morinda tinctoria.*

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliotae
Order:	Gentianales
Family:	Rubiaceae
Genus:	*Morinda*
Species:	*Tinctoria*

Traditional uses of *Morinda tinctoria.*

The leaves and root of *Morinda tinctoria* are used as astringent and pain relievers in a cute inflammatory arthritis leading to gout. *Morinda tinctoria* leavespossess anticonvulsant, analgesic, anti-inflammatory, antioxidant activity and antimicrobial properties. Orally given leaf juice is useful for easy digestion of food specially in children. Fruit juice is given orally for diabetes and cardiovascular diseases. In the traditional system of medicine, leaves and roots of *Morinda tinctoria* are used as astringent, deobstrent, emmengogue and to relieve pain in the gout. It has been proved that leaves and root of *Morinda tinctoria* have therapeutic and nutritional values. There is a greater demand for fruit extract of morinda species in treatment for different kinds of ailments like cancer, arthritis, heart diseases and gastric ulcer. In controlling ammonia pollution in waste water ashes of leaves of Morinda *tinctoria used* as bio-sorbent [12].

I. Material And Methods

The fresh leaves of Morinda tinctoria, are collected from Kada, District Beed. The fresh leaves were dried not in sunlight but in shade. Then it was grinded and made a fine powder. It was passed through 40 mesh sieve and stored in an air-tight bottle to be used further. With different solvents like ethanol, water, acetone, petroleum ether and chloroform this powder was extracted by Soxhlet apparatus.

Ash Analysis:

Ash value is helpful in determining the quality and purity of crude drug, is especially in powder form [13-15].

Extractive Value:

Extractive value of crude drug is useful for their evaluation, especially when the constituents of a drug cannot be readily estimated by any others means. Further, these values indicate the nature of constituents present in crude drug [16].

Phytochemical Analysis:

Phytochemical examinations were carried out for all the extract as per the standard methods [17].

Metal Detection:

The samples were sent to Dr. Baldeo Singh senior scientist Indian institute of integrative medicine (CSIR), Jamu-Tawi for metal content. The metal present in sample were detected by inductive coupling plasma spectroscopy which is superior than double atomic absorption spectroscopy.

II. Resultand Discussion

In present investigation sample leaves of Morinda tinctoria was taken and burnt complectely in presence of oxygen. The ash was weighed till constant weight is obtained. The total ash obtained is 30%. Further the ash is treated with HCl to obtain acid insoluble value which is found to be 28 %. Similarly, water soluble ash is calculated which is found to be 72.1%. The presence of ash indicates inorganic material present in it [Table.1].

Leaves of Morinda tinctoria extracted with different solvent such as water, ethanol, chloroform and acetone and petroleum ether. The extractive value in water is found to be 19.14 %, extractive value of ethanol is found to be

21.18 %, chloroform extractive value is 15.06 %, acetone extractive value is 13.14 % and extractive value of petroleum ether is found to be 10.11% [Table.2].

Present investigation shows the presence of alkaloid, saponin, flavonoid and amino acid in aqueous extract. Ethanolic and chloroform extract shows the presence of carbohydrate, alkaloid, glycoside, phenol, flavonoid, protein and amino acid, while acetone and petroleum ether extract show presence of alkaloids, glycoside, flavonoids [Table.3].

Antimicrobial activity of Morinda tinctoria leaves in different solvent was carried out. In aqueous extract zone of inhibition is 3 mm for Proteus vulgaris and B. megaterium. Ethanol extract shows zone of inhibition 1-5 mm. Chloroform extract shows 1mm zone of inhibition against P. aeruginosa. Acetone extract shows zone of inhibition 5-8mm. Petroleum ether extract shows no zone of inhibition [Table.4].

Antimicrobial activity of various extract of leaf of Morinda tinctoria [18] shows zone of inhibition which varies in between 12-18 mm. The study also concludes that Morinda tinctoria leaves contain a number of pharmaceutically important phytochemicals like alkaloids, saponins, carbohydrates, phenol, tannin and flavonoids. These bioactive compounds are responsible for potent antimicrobial activity.

UV-visible spectral analysis of Morinda tinctoria inaqueous extract shows presence of eight peaks and its max observed at 208 nm. Ethanolic extract of it shows maximum twenty-four peaks and max at 226 nm. Chloroform extract shows number of peaks eleven and max observed at 248 nm. Acetone extract of Morinda tinctoria shows max at 675 nm and number of peaks shown by itare 16. Petroleum ether extracts shows presence of 6 peaks and its max observed at 212 nm. Therefore, each solvent extract different compounds [Table.5].

Aqueous extract of Morinda tinctoria shows absorption band found at 3264 cm^{-1} due to O-H stretching and 2922 cm^{-1} due to C-H stretching. The band found at 1398 cm^{-1} and 1578 cm^{-1} due to symmetric and asymmetric stretching of $-NO_2$ group with benzene ring. The band at 1076 cm^{-1} and 874 cm^{-1} is due to C-O and p-substituted benzene ring.

Ethanolic extract shows absorption band at 3303 cm^{-1} due to O-H stretching and 2917 cm^{-1} and 2849 cm^{-1} indicates there is C-H stretch. The band at 1607 cm^{-1} and1538 cm^{-1} confirms presence of benzene ring. The band at 1323 cm^{-1} indicates presence of $-NO_2$ group. The band found at 1023 cm^{-1} due to C-O group.

The chloroform extract shows absorption band at 2916 cm^{-1} and 2848 cm^{-1} due to C-H stretching. The band at 1618 cm^{-1} and 1462 cm^{-1} due to C=C of benzene ring. The band found at 1035 cm^{-1} and 719 cm^{-1} due to C-O and C-Cl group.

Acetone extracts shows presence of 2916 cm^{-1} and 2848 cm^{-1} due to C-H stretching. The band at 1731 cm^{-1} and 1611 cm^{-1} due to C=C of benzene ring. The bands at 1362 cm^{-1} due to $-NO_2$ group and 719 cm^{-1} due to C-Cl stretching.

Petroleum ether extract shows absorption band at 2917 cm^{-1} and 2848 cm^{-1} is due to C-H stretching. The band at 1731 cm^{-1}, 1614 cm^{-1} and 1462 cm^{-1} is due to C=O, C=C of benzene ring. The absorption band at 1034 cm^{-1} and 719 cm^{-1} is due to C-O and C-Cl stretching [Table.6].

Morinda tinctoria evaluated for nickel, copper, cobalt and iron content. It shows 20.0 ppm of nickel, 82.6 ppm of copper, 4.1 ppm of cobalt and 8154.9 ppm of iron. The trend is similar as earlier Fe > Cu > Ni > Co. The highest concentration of iron was observed in Morinda tinctoria.

Table 1: Ash analysis of *Morinda tinctoria* leaves.

Sr. No.	Type of ash	Percentage(w/w)
1	Total ash	30.0 %
2	Acid insoluble ash	28.0 %
3	Water soluble ash	72.1%

Table 2: Percentage extractive value of *Morinda tinctoria* leaves.

Sr. No.	Type of extractive value	Percentage(w/w)
1	Water	19.14 %
2	Ethanol	21.18 %
3	Chloroform	15.06 %
4	Acetone	13.14 %
5	Petroleum ether	10.11%

Table:3 Phytochemicals present in different extract of *Morinda tinctoria* leaves.

(+ Present& - Absent)

Phytochemicals	Carbohydrates	Alkaloid	Glycosides	Saponin	Phytosterol	Phenol	Tannin	Flavonoid	Protein & amino acid
Water	-	+	+	+	-	-	-	+	+
Ethanol	+	+	+	-	+	+	-	+	+
Chloroform	+	+	+	+	-	+	-	+	+
Acetone	-	+	+	-	-	+	-	+	+
Petroleum ether	-	+	+	+	-	-	-	+	-

Table 4: Antibacterial activity of *Morinda tinctoria* leaves in different solvent.

Sr. No.	Name of organism	Aqueous extract (mm)	Ethanol extract (mm)	Chloroform extract (mm)	Acetone extract (mm)	Petroleum ether extract (mm)
1	*Staphylococcus aureus*	-	5	-	8	-
2	*Salmonella typhimurium*	-	1	-	5	-
3	*Proteus vulgaris*	3	4	-	6	-
4	*Pseudomonas aeruginosa*	-	-	1	-	-
5	*B. megaterium*	3	-	-	5	-

Table 5:Absorption peaks in UV-Visible range of *Morinda tinctoria* for different extracts.

Sr. No.	Aqueous extract		Ethanol extract		Chloroform extract		Acetone extract		Petroleum ether extract	
	λ (nm)	**O.D.**	**λ (nm)**	**O. D.**	**λ (nm)**	**O.D.**	**λ (nm)**	**O.D.**	**λ (nm)**	**O.D.**
1	208	5.94	204	3.51	248	4.65	675	4.97	212	6.22
2	210	5.79	226	4.47	255	4.51	542	4.93	217	5.00
3	224	4.61	214	3.96	674	4.49	333	4.52	427	4.67
4	227	4.44	219	4.21	281	4.15	546	4.46	481	4.48
5	221	4.22	241	4.59	289	4.15	549	4.09	423	4.07
6	374	4.07	277	4.38	345	4.11	668	4.13	659	4.04
7	432	2.42	-	-	365	4.10	378	4.13	-	-
8	-	-	-	-	-	-	372	4.13	-	-

Table 6:I. R. Peak value of *Morinda tinctoria* for different extract.

Sr. No.	Extracts	I. R. Observed peaks (cm^{-1})
1	Water	3264, 2922, 1578, 1398, 1076, 1020, 874.
2	Ethanol	3303, 2917, 2849, 1607, 1538, 1444, 1323, 1203, 1032.
3	Chloroform	2916, 2848, 1618, 1462, 1035, 1004, 719.
4	Acetone	2916, 2848, 1731, 1611, 1462, 1364, 1202, 1035, 719, 619.
5	Petroleum ether	2917, 2848, 1731, 1614, 1462, 1376, 1242, 1034, 1007, 719.

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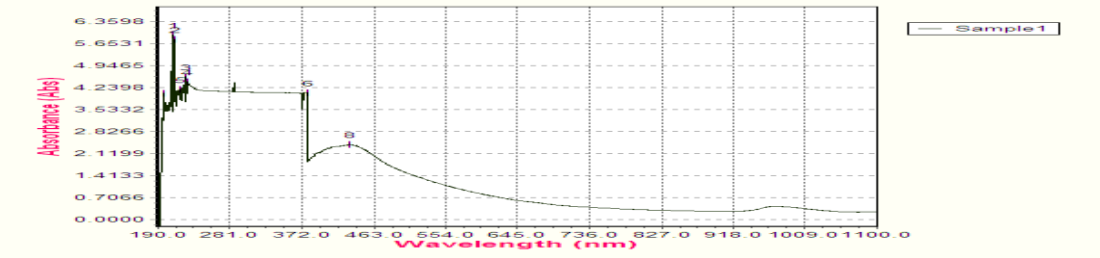
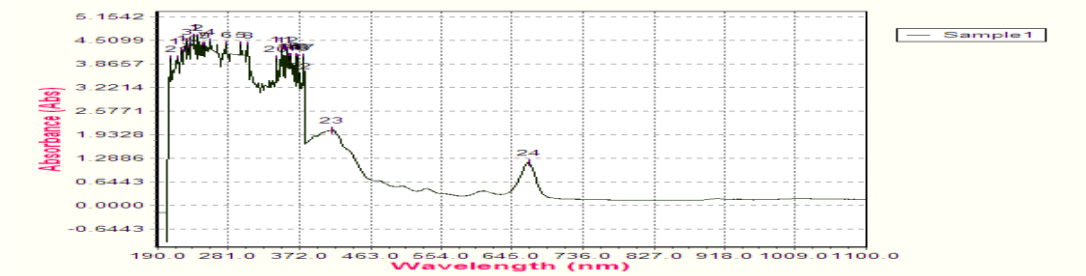
Figure 1:*UV spectra of Morinda tinctoria for various extract.*
(a) Water Extract (b)Ethanol Extract (c) Chloroform Extrct (d) Acetone Extract and (e) Petroleum ether Exxtract.

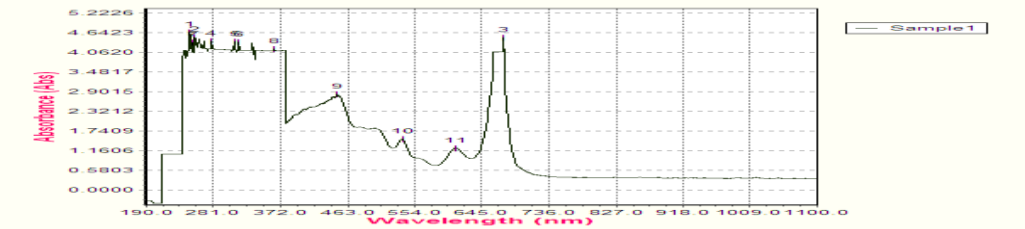
(a)



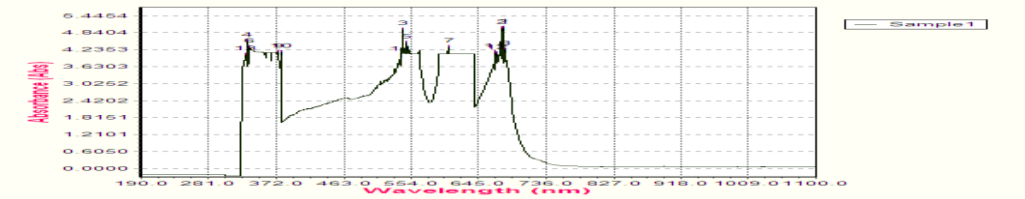
(b)



(C)



(d)



(e)

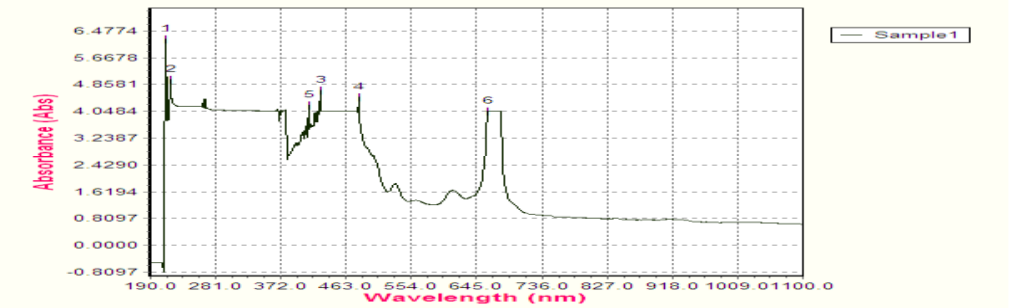
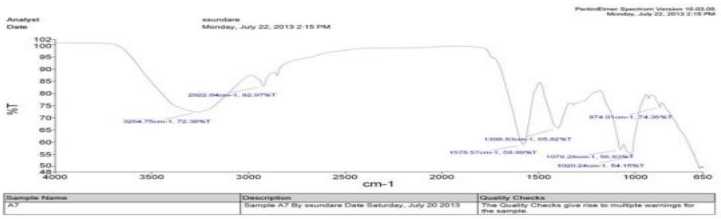
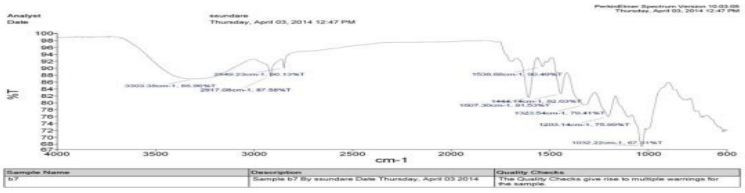


Figure 2: IR spectra of Morinda tinctoria for various extract.
(a) Water Extract (b)Ethanol Extract (c) Chloroform Extrct (d) Acetone Extract and (e) Petroleum ether Exxtract.

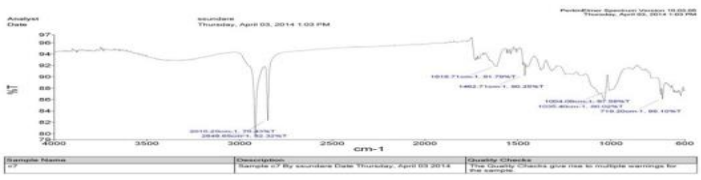
(a)



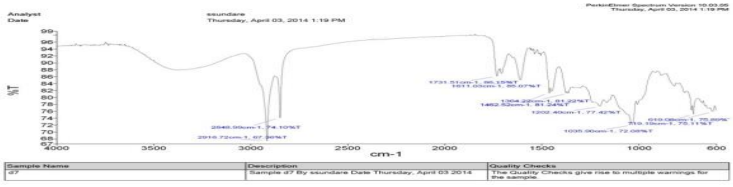
(b)



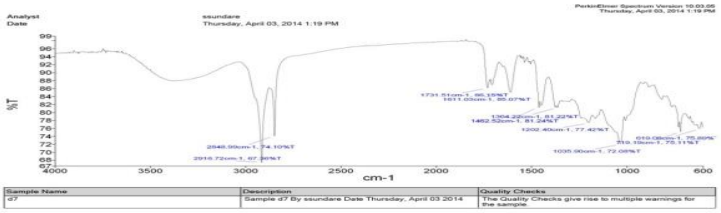
(c)



(d)



(e)



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