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PRELIMINARY PHYTOCHEMICAL STUDIES OF MORINDA TINCTORIA LEAF EXTRACT

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Abstract:

Objectives: *To evaluate the phytoconstituent of various extract of Morinda Tinctoria leaf Morinda tinctoria, commonly known as aal or Indian mulberry (though these common names also refer to Morinda citrifolia), is a species of flowering plant in the family Rubiaceae, native to southern Asia.The plant material may be subjected to preliminary phytochemical screening for the detection of various plants constiuents. Extraction of aqeous, ethanolic and acetone solvent to be used.*

Conclusion: *It can be concluded that the source of secondary metabolites like flavonoids, carbohydrates, glycosides, alkaloids, phenols and phytosterols are present in the morinda Tinctoria leaf.*

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INTRODUCTION:

The plant may be considered as a biosynthetic laboratory not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as volatile oils, Tannins etc., that exerts a physiological effect. The compounds that are reponsible for theraputic effect are usualy the secondary metabolites. A systemic study of a crude drug embraces through consideration of both primary and secondary metabolites derived as a result of plant metabolism. The plant material may be subjected to preliminary phytochemical screening for the detection of various plants constituents.

For our present study, we had taken the plant material as powdered leaves of *Morinda tinctoria* to extract the compounds are tested the chemical constituents present in them.

Morinda tinctoria, commonly known as aal or Indian mulberry (though these common names also refer to Morinda citrifolia), is a species of flowering plant in the family Rubiaceae, native to southern Asia. It is 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 scarp, 2-2.5 cm diameter. Distributed in all tropical regions of the world.

Purification of solvents:

Ethanol:

A dry round bottom flask was fitted with a double surface condenser and a calcium chloride guard tube. Dry magnesium turnings (5gm) and iodine (0.5gm) were placed in the flask followed by 50-75ml of commercial absolute alcohol. The mixture was warmed until the magnesium is converted to ethanolate, then 900ml of commercial absolute alcohol was added and refluxed for 30minutes. The ethanol is directly distillled into vessel and used.

Distilled Water:

Water obtained by distillation is used aqueous extraction of powdered drug material.

Preparation of extracts:

Prepration of the extract of powdered leaves of Morinda Tinctoria is done by using following solvents,

(a) Aqueous extract
(b) Ethanolic extract
(c) Acetone extract

Aqueous extract:

The shade dried coarse powder of leaf (100 mg) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with 500ml distilled water for 24 hours. The extract was distilled in vacuum under pressure in order to remove the solvent completely. It was dried and kept in a decicator till experimentation. Obtained extract was weighed and % yield was calculated in terms of air-dried powdered crude material.

Ethanoic extract:

The shade dried coarse powder of leaf (100 mg) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with 500ml of absolute alcohol for 24 hrs The extract was distilled in vacuum under pressure in order to remove the solvent completely. It was dried and kept in a decicator till experimentation. Obtained extract was weighed and % yield was calculated in terms of air-dried powdered crude material.

Acetone extract:

The shade dried coarse powder of leaf (100 mg) was packed well in soxhlet apparatus and was subjected to continuous hot extraction with 500ml acetone for 24 hrs The extract was distilled in vacuum under pressure in order to remove the solvent completely. It was dried and kept in a decicator till experimentation. Obtained extract was weighed and % yield was calculated in terms of air-dried powdered crude material.

The yeild and %yeild of various extracts of powdered leaves of *Morinda Tinctoria* were reported in the table no: 1

Table no: 1 Extractives values of aqueous extract, Ethanoic extract and Acetone extracts of powdered leaves of *Morinda Tinctoria*

Sr.no	Extracts	%Yield(w/w)
1.	Aqueous extract	24
2.	Ethanolic extract	17
3.	Acetone extract	14

QUALITATIVE PHYTOCHEMICAL ANALYSIS:

Both ethanolic and aqueous exatracts obtained from the *Morinda Tinctoria* was subjected to various qualitative test for the identification of various plant constituents present in species.

Test for Alkaloids:

(a) Dragendroff's Test: To 1 ml of extract, add 1ml of Dragendroff's Reagent (potassium bismuth iodide solution). An orange-red precipitate indicates the present of alkaloids.

(b) Mayer's Test: To 1ml of extract, add 1ml of Mayer's reagnt (potassium mercuric iodide solution). Whitish yellow or cream-coloured precipitate indicates the presence of alkaloids.

(c) Hager's Test: To 1ml of extract, add 3ml of Hager's reagent (saturated petroleum ether solution of picric acid), yellow colored precipitate indicates the presence of alkaloids.

(d) Wagner's Test: To 1ml of extract, add 2ml of Wagner's reagent (iodine in potassium iodide), Formation of reddish brown precipitate indicates the presence of alkaloids.

Test for Saponins:

Take small quality of ethanolic and aqueous extracts seprately and add 20ml of distilled water and shaken in graduated cylinder for 15 minutes lengthwish. A 1cm of distilled water and shaken in graduated cylinder for 15 minutes lengthwise. A1cm layer of foam indicates the presence of saponins.

Test for Glycosides:

a) **Legal Test:** Dissolve the extract in pyridine and add sodium nitroprusside solution to make it alkaine. The formation of pink red to red color shows the presence of glycosides.

b) **Baljet Test:** To 1ml of the extract, add 1ml of sodium picrate solution and the yellow to orange color reveals the presence of glycosides.

c) **Keller-Killiani Test:** 1ml of powdered drug is extracted with 10ml of 70% alcohol for 2 minutes, filtered, add to the filtrate, 10ml of water and 0.5ml of strong solution of lead acetate and filtered and the filterate is shaken with 5ml of chloroform. The chloroform layer is separated in porcelain dish and removes the solvent by gentle evaporation. Dissolve the cooled residue in 3ml of glacial acetic acld contining 2 drops of 5% ferric chloride solution. Carefully transfer this solution to the surface of 2ml of concentrated sulphuric acid. A reddish-brown layer forms at the junction of the two liquids and the upper layer slowly becomes bluish green, darkening with standing.

d) **Borntrger's Test:** Add a few ml of dilute sulphuric acid to 1ml of the extract solution. Boil, filter and extract the filtrate with chloroform .the chloroform layer was treated with 1ml of ammonia. The formation of red color of the ammonical layer shows the presence of anthraquinone glycosides.

Test for Carbohydrates:

a) **Molisch's Test:** To 2ml of the extract, add 1ml of α-napthol solution, add concentrated sulphuric acid through the through the side of the test tube. Purple or reddish violet colour at the junction of the two liquids reveals the presence of carbohydrates.

b) **Fehling's Test:** To 1ml of extact, add equal quantities of Fehling's solution A and B, upon heating formation of a brick red precipitate indicates the presence of sugars.

c) **Benedict's Test:** To 5ml of Benedict's reagent add 1ml of extract solution and boil for 2 minutes and cool. Formation of red precipitate shows the presence of sugars.

d) **Test for Tannins**

e) Take the little quality of test solution and mixed with basic lead acetate solution formation of white precipitates indicates the presence of tannins.

f) To 1ml of the extract, add ferric chloride solution, formation of a dark blue or greenish blank color product shows the presence of tannins.

g) The little quantity of test extract is treated with potassium ferric cyanide and ammonia solution. A deep red color indicates the presecnce of tannins.

h) To the test extract, add strong potassium dichromate solution, a yellow color precipitate indicates the presence of tannins and phenolics.

Test for Flavonoids:

a) The drug in ethanolic and aqueous solution with few ml of ammonia is seen in U.V and visible lighit; formation of fluorescence indicates the presence of flavonoids.

b) Little quality of extract is treated with amyl alcohol, sodium acetate and ferric chloride. A yellow color solution formed, disappears on addition of an acid indicetes the presence of flavonoids.

c) **Shinoda's Test:** The ethanolic and aqueous extracts of power treated with magnesium foil and concentrated HCL gives intence cherry red color indicates the presence of flavanones or orange red color indicates the presence of flavonols.

d) The extract is treated with sodium hydroxide, formation of yellow color indicates the presence of flavones.

e) The extract is treated with Concentrated H_2SO_4, formation of yellow or orange color indicates flavones.

f) The ethanolic and aqueous extracts were treated with 10% sodium chloride; formation of yellow color indicates the presence of coumarins.

Test for Phyto-sterols:

a) **Libermann-Burchard Test:** 1gm of the test substance was dissolved in a few drops of chloroform, 3ml of acetic anhydride, 3ml of glacial acetic acid were added, warmed and cooled under the top and drops of concentrated sulphuric acid were added along the sides of the test tube. Appearance of bluish-green color shows the presence of sterols.

b) **Salkowski Test:** Dissolve the extract in chloroform layer and add equal layer and green fluroescence in the acid layer represents the steroidal compounents in the tested extract.

Test for proteins and Amino Acids:

a) **Biuret Test:** Add 1ml of 40% sodium hydroxide solution and 2drops of 1% $CuSO_4$ solution till a blue colour is produced, then add to the 1ml of the extract, Formation of pinkish or purple violet colour indicates the presence of proteins.

b) **Ninhydrin Test:** Add two drops of freshly prepared 0.2% Ninhydrin regent (0.1% solution in n-butanol) to the small quantity of extract solution and heat. Development of blue color reveals the presence of proteins, peptides or amino acids.

c) **Xanthoproteic Test:** To 1ml of extract, add 1ml of concentrated nitric acid. A white precipitate formed, it was boiled and cooled. Then 20% of sodium hydroxide or ammonia is added. Orange color indicates the presence of aromatic ammino acids.

d) **Millon's Test: 1**ml of test solution is made acidify with sulphuric acid and add Millon's reagent and boil this solution. A yellow precipitate is formed indicates the presence of protein.

Test for Triterpenoids:

a) **Noller's Test: D**issolve two or three granules or tin metal in 2ml tinonyl chloride solution. Then add 1ml of the extract into test tube and warm, the formation of pink color indicates the presence of triterpenoids.

b) **Salkowski's test:** Equal quality of chloroform is treated with plant extract and filtered with few drops of conc.H_2SO_4 and shaken well and allowed to stand. Golden yellow layer at the bottom indicates the presence of triterpenoids.

Test for Fixed Oils and Fats:

a) **Spot Test:** Press a small quality of extracts between the filter paper. Oil stains on paper indicates the presence of fixed Oils.

b) **Saponification Test:** To 1ml of the extracts, add few drops 0.5N alcoholic potassium hydroxide along with a drop of phenophthalein. Heat the mixture on a water bath for 1-2 hours. The formation of soap or partial neutraization of alkali indicates the presence of fixed oils and fats.

Test for Gums and Mucilage:

a) Add about 10ml of aqqueous extract slowly to 25ml of absolute alcohol with constant stirring. Filter the precipitate and dry in air. Examine the precipitate for its swelling properties and for the presence of carbohydrates.

Test for Lignins:
With alcoholic solution of phloroglucinol and hydrochloric acid, the appearance of red color shows the presence of lignins.
(a)Labat test Extract solution with galic acid to form an olive green colour shows the presence of lignins.
(b) Furfuraldehyde test Extract solution with 2% furfuraldehyde solution appears a red colour shows the presence of lignins.

Test for Cholestrol:
Add 2ml of plant extract with 2ml chloroform with 10 drops of acetic anhydride add 2-3drops of conc.H_2SO_4 appears a red-rose colour shows the presence of cholestrol.

Test for Terpinoides:
Add 2ml of choloroform with 5ml of plant extract, (evaporated on water bath) add 3ml of conc.H_2SO_4 (boiled on water bath). Appears A grey colourrd solution shows the presence of terpinoides.

Test for Diterpenes:
Plant Extract is dissolved in distilled water and add 3-4 drops of copper acetate solution appears Emerald green colour it shows the presence of diterpenes.

Test for Carotenoids:
(a) Carr-Price Reaction
Add 10ml extract evaporated to dryness and add 2-3 drops of saturated solution of atimony trichloride in chloroform appears A blue-green colour eventually changing to red. shows the presence of carotenoids.

Test for Quinones:

a) Alcoholic KOH test : Add 1ml plant extract with few ml alcoholic potassium hydroxide appears Red to blue colour shows the presence of Quinones

b) Conc. HCL test: Plant extract with conc.HCL appears a green colour it shows the presence of Quinones.

c) **Sulphuric acid test:** Add 10mg of extract dissolved in isopropyl alcohol with 2 drops of sulphuric acid appears A red colour it shows the presence of Quinones.

Test for Anthraquinones:

(a) Borntrager; s test Add 10ml 10% ammonia soluion with few ml of 3ml of aq. Extract is shaken with 3ml of benzene and filtered(shaken vigorously for 30sec.) appears A pink,violet,or red coloured solution it shows the presence of anthraquinones.

(b) Ammonium hydroxide test Add 10mg extract is dissolved in isopropyl alcohol is a drop of conc. ammonium hydroxide solution appears formation of red colour after 2minutes it shows presence of anthraquinones.

Test for Leuconthocyanins:

a) Isoamyl alcohol test
Add 5ml plant extract with 5ml isoamyl alcohol appears upper layer appears red it shows the presence of Leuconthocyanins.

b) Test for Carboxylic acid
Effervesence test: Add 1ml plant extract with 1ml sodium bicarbonate solution it appearance of Effervesence it shows the presence of carboxylic acid.

S.NO	PHYTO CONSTITUENTS	AQUEOUS	ETHANOLIC	ACETONE
1.	Alkaloids	-	+	+
2.	Cardiac Glycosides	-	+	+
3.	Saponin Glycosides	-	-	-
4.	Coumarin Glycoside	+	+	+
5.	Tannis	-	+	+
6.	Phlobatannin	-	+	+
7.	Phytosterol	+	+	+
8.	Cholestrol	+	-	+
9.	Terpinoids	-	-	-
10.	Diterpinoids	+	+	+
11.	Triterpenoids	+	+	-
12.	Lignins	-	+	+
13.	Carotenoids	-	-	-
14.	Anthraquinones	-	-	-
15.	Anthocyanins	-	-	-
16.	Leuconthocyanins	+	-	-
17.	Carboxylic Acids	-	-	-
18.	Flavonoids	+	+	+
19.	Phenolic Compounds	+	+	+
20.	Gum And Mucilage	-	-	-
21.	Resins	-	-	-

CONCLUSION:

It can be concluded that the source of secondary metabolites like flavonoids, carbohydrates, glycosides, alkaloids, phenols and phytosterols are present in the morinda Tinctoria leaf. As a result, for more reliable results, two or more separate Tests should be performed.L

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