

EXTRACTION AND PRELIMINARY PHYTOCHEMICAL SCREENING OF ACTIVE COMPOUNDS IN *MORINDA CITRIFOLIA* FRUIT

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ABSTRACT

The study was designed to investigate the phytochemical screening of three different extract of *Morinda citrifolia* Linn Rubiaceae. The crude extracts obtained from the fruit of *Morinda citrifolia* in different solvents ethanol, methanol and aqueous extract were subjected to phytochemical study. Phytochemical studies indicated that *Morinda citrifolia* contained a broad spectrum of secondary metabolites. Alkaloids, saponins and reducing sugar were predominantly found in all the three tested extracts followed by steroid, phenol, tannin and terpenoids. Likewise cardiac glycoside, carbohydrate and flavanoids were found in all the tested solvents and aqueous extract of the fruit. Protein is found in aqueous and ethanol extract and it is absent in methanol extract. Resin, anthraquinone, phylobatannin were not found in any of the extracts of *Morinda citrifolia* fruit. The present study clearly demonstrates the extraction and phytochemical screening of *Morinda citrifolia* fruit.

Keywords: *Morinda citrifolia*, fruit, extract, phytochemical screening, aqueous and solvent extract.

INTRODUCTION

India is known for its rich diversity of medicinal plants and from ancient times these plants were utilized as therapeutic agents. Today's research is mainly focused on medicinal plants because the bioactive compounds and medicinal power mainly depends on phytochemical constituents that have great pharmacological significance. The phytochemical constituents, natural bioactive compounds, nutrients and fibers present in medicinal plants, fruits and vegetables defend us from various ailments¹. Phytochemicals are classified into two major groups namely primary constituents like amino acids, sugars, proteins and chlorophyll etc., secondary constituents includes alkaloids, essential oils, flavanoids, tannins, terpenoids, saponins and phenolic compounds etc., more over they bear valuable therapeutic activities². During last few decades, high bioactive compounds were identified and screened from many plant species. *Morinda citrifolia* is an important medicinal plant with rich source of phytochemical constituents.

Morinda citrifolia Linn Rubiaceae is one of the most important traditional Polynesian medicinal plant commercially known as Noni, Indian mulberry, Ba ji Tian, Nono or Nonu, Cheese fruit and Nhau in various cultures throughout the world, indigenously found in open coast region at sea level and in forest areas about 1,300 feet above sea level, it is a small tropical evergreen shrub or tree, three to twelve meters height it has straight trunk, large green leaves and distinctive grenade like yellow fruit. The size of fruit is about 12cm due to coalescence of the interior ovaries of many closely packed flowers; it has a foul taste and a soapy smell when ripe. The fruit of this plant has been used as food, drink, medicine, colorful dye, cosmetics purpose and has a high demand in medicines for different kinds of illnesses like diabetes, high blood pressure, AIDS, arthritis, cancer, gastric ulcer, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problem etc., Its root, leaves, stem, bark, flowers and fruits are recorded as herbal remedies for different diseases³⁻⁴.

Morinda is reputed to have antibacterial, antiviral, antifungal, antitumor, anti tubercular effect, analgesic activity, immunological activity, mental health and improve high frequency, antihelminthic, analgesic, hypotensive, anti inflammatory, immune enhancing etc., due to its beneficial effects, the fruit juice of *Morinda citrifolia* is widely distributed throughout the world as nutraceutical dietary supplement. The leaf of this plant is directly used on skin for ulcerations and for minor infections⁵⁻⁶. The aim of the present study is to extract and to analyze the phytochemicals present in *Morinda citrifolia* fruit

The fruits of *Morinda citrifolia* were collected from World Noni Research Foundation, Perungudi, Chennai. The fresh fruits were

washed with tap water for several times chopped with sterile knife and dried in Hot air oven at 45°C for 48 hours. Air dried fruit is grounded into powder; the grounded powder was extracted with methanol, ethyl alcohol and incubated for 72 hours in shaker, where as the aqueous extract was prepared by incubating for overnight in shaker and it was boiled for 15 to 30 minutes till the volume was reduced to half its original. The solvent was then removed by filtration. The extracts were condensed using rotary vacuum evaporator and stored at 0-4°C. The aqueous extract was dissolved in water and used for further purpose whereas concentrated tonic extract of ethanol and methanol was suspended in 0.25% dimethyl sulphoxide (DMSO) to the concentration of 100mg/ml and was used for analysis.

The aqueous, ethanol and methanol extracts of *Morinda citrifolia* were screened for the presence of secondary metabolite using the standard procedure. Three milliliter (ml) of each extract was measured into a test tube for each test. Tests were carried out for Steroid, Cardiac glycoside, Phenol, Tannin, Terpenoid, Alkaloids, Resins, Carbohydrate, Flavanoids, Anthraquinone, Phylobatannin, Reducing Sugar, Saponin, proteins, Lipids and fats, Acidic components according to standard methods⁷⁻¹⁰.

Steroid (Liebermann Burchard reaction): 300µl of extract was added with 1ml of chloroform and few drops of Concentrated Sulphuric acid along the sides of the test tubes, Reddish brown color precipitate was observed at the bottom of the test tubes indicates the presence of steroid.

Cardiac Glycoside (Keller Kiliani Test): 300µl of extract was added with 1ml of Acetic acid followed by the addition of 300µl of 10% Ferric Chloride and few drops of Concentrated Sulphuric acid along the sides of the test tubes, Brownish ring and green blue precipitate at the bottom of the test tube indicates the presence of Cardiac glycoside.

Phenol and Tannin (Ferric Chloride Test): Few drops of Ferric chloride 10% was added with 300µl of extract gives Blue or Green color precipitate due to the presence of Phenol and Tannin.

Terpenoids (Salkowski Test): To 300µl of extract 1ml of chloroform and few drops of Concentrated Sulphuric acid was carefully added along the sides of the test tubes, a reddish brown color precipitate indicates the presence of Terpenoid.

Alkaloids (Mayer's Test): 300µl of Mayer's reagent was added with 300µl of fruit extract. Pale precipitate indicates the presence of Alkaloids.

Resins: To 300µl of extract 1ml of acetic anhydride was added and dissolved by gentle heating. After cooling, few drops of concentrated sulphuric acid were added. Bright purple color indicates the presence of Resin.

Carbohydrate (Molisch's Test): Few drops of sulphuric acid and 300µl of Molisch's reagent were added with 300µl of extract, reddish color at the bottom of the test tubes indicates the presence of Carbohydrates.

Flavanoids: 300µl of extract was first added with 1ml of 10% ammonia and 1ml of concentrated sulphuric acid. Disappearance of yellow color indicates the presence of Flavanoids.

Anthraquinones (Borntrager's Test): 1ml of benzene and 1ml of 10% ammonia was added with 300µl of extract. Presence of anthraquinone was observed by the formation of pink, red or violet color in the lower phase of ammonia.

Reducing sugar: Few drops of Molisch's reagent were added with dilute extracts and heated for 30 minutes and observed for the formation of brick red colored precipitate.

Saponins (Froth Test): 300µl of extract was added with 2ml of distilled water in a test tube. The solution was vigorously shaken and observed for the stable froth persistence.

Protein (Nin hydrin Test): 1ml of distilled water was added with 300µl of extract and 300µl of Nin hydrin. The solution was boiled for 5 to 10 minutes. Dark purple color indicates the presence of Amino acids.

Lipids and Fat: A small quantity of powdered drug was rubbed on a clean and neat filter paper and observed for a permanent translucent strain.

Acidic compounds: one pinch of sodium bicarbonate was added with 300µl of extract. Effervescence indicates the presence of acidic compounds. Two duplicates are maintained for each test.

Table 1: phytochemical screening of fruit extract of *morinda citrifolia*

Phytochemical	Inference		
	Aqueous	Methanol	Ethanol
Steroids	++	++	++
Cardiac Glycosides	++	++	++
Phenol	+	++	++
Tannins	++	+	++
Terpenoids	++	++	++
Alkaloids	+	++	+
Resins	-	-	-
Carbohydrates	++	++	++
Flavanoids	++	++	++
Anthraquinones	-	-	-
Phylobatannins	-	-	-
Reducing sugar	+	+	++
Saponins	+	+	-
protein	++	-	++
Lipids and fats	+	+	+
Acidic Compounds	+	-	-

- = Absent, + = Present, ++ = Appreciable amount.

Preliminary phytochemical screening of *Morinda citrifolia* fruit extracts revealed the presence of various bioactive compounds and the results were summarized in Table 1. The phytochemical screenings of *Morinda citrifolia* fruit extracts of these three solvents are more or less similar. The phytochemical analysis of *Morinda citrifolia* shows the presence of all major phytochemicals of steroid, cardiac glycosides, terpenoids, carbohydrates and flavanoids in appreciable amount and other components in moderate amount. Whereas phylobatannins, anthraquinones and resins are absent.

Due to the modern civilization the resources of medicinal herbs are dwindling fast. Though, the purified plant chemicals are obtained from a significant number of studies only very few screening programs are initiated on crude plants. It is also accepted that the

medicinal value of plants depends on the bioactive phytochemicals in plants associated to antibacterial activities and also have a curative property against pathogens. Therefore now there is a need to look back towards the traditional medicines which can serve as novel therapeutic agents¹¹.

Steroids are known to be an important Cardio tonic activities posse's antimicrobial property and also used in herbal medicines and cosmetics. The presences of cardiac glycosides are known to play a major role in heart muscles by inhibiting Na⁺ and K⁺ pump that increase the availability of sodium ions and calcium ions to heart muscles which improves cardiac output and reduce heart distension. Thus are used in the treatment of congestive heart failure and cardiac arrhythmia¹². Flavanoids and tannin are the group of phenolic compounds that act as primary antioxidants and posse's antimicrobial, anti-inflammatory, antiallergic, anticancer, antineoplastic activity, and for the treatment of intestinal disorders¹³. Alkaloids are one of the largest groups of phytochemicals that have led to the invention of powerful pain killer medications¹⁴. Saponins which act as bioactive antibacterial agents in plants are also used to treat hypercholesterolemia, hyperglycemia and obesity¹⁵.

Preliminary phytochemical surveys and the knowledge of the chemical constituents of plants are desirable to understand herbal drugs and their preparations. Therefore, the phytochemical investigation of *Morinda citrifolia* fruit in the present study reveals the presence of various potential phytochemical constituents which may be useful for pharmaceutical industries and could be used as an effective nutraceutical. However, further studies are needed to isolate and purify the bioactive compound of this useful traditional plant *Morinda citrifolia* for industrial drug formulation.

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