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Research Article

PHYSICOCHEMICAL, PHYTOCHEMICAL AND ANTIMICROBIAL STUDIES ON *MORINDA CITRIFOLIA* L. FRUITS AT DIFFERENT MATURITY STAGES

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ABSTRACT

The present investigation was designed to evaluate the physicochemical, phytochemical and antimicrobial properties of various maturity stages of *Morinda citrifolia* L. fruit extracts. Physicochemical parameters such as total ash, water soluble ash, acid insoluble ash, loss on drying and extractive values were determined. Fruits were extracted with ethanol, methanol, chloroform, ethyl acetate and water to analyse the presence of phytochemicals such as, carbohydrate, protein, tannin, flavonoid, saponin, steroids, alkaloids and glycosides. The antimicrobial properties of various extracts were assayed using the agar well diffusion method against five strains namely *Escherichia coli*, *Klebsiella pneumoniae, Salmonella typhi*, *Aspergillus niger* and *Aspergillus flavus.* The maximum antibacterial activity was found against *K. pneumonia* (21 mm) in ethanol extract of mature *M. citrifolia* fruits whereas maximum antifungal activity was found against *A. flavus* (19 mm) in mature methanol extract.

Keywords: *Morinda citrifolia,* Maturity stages, Phytochemical analysis, Antimicrobial activity.

INTRODUCTION

Medicinal plants are renowned natural sources for the treatment of various ailments since prehistoric times. Many important drugs have been directly or indirectly derived from them[1]. Plant derived drugs remains important resource in developing countries, to combat serious disease[2]. According to the World Health Organization (WHO) more than 80% of the world's population relies on the traditional medicine for the primary health care needs[3]. Medicinal plant remains the source of inspiration of novel drug compounds as they afford key chemical structure for the progress of new antimicrobial drugs as well as phytomedicine[4]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body[5]. The most important bioactive compounds of plant are alkaloids, flavonoids, tannins and phenolic compounds[6]. These phytochemicals are antibiotic principles of plants and have been reported to possess anti-bacterial, anti-fungal and anti-inflammatory activities[7]. Thus, medicinal plants play an important role in developing of newer drugs because of their effectiveness, less side effects and relatively low cost when comparing with synthetic drugs[8].

Morinda citrifolia L (Rubiaceae), commonly known as Noni, is an evergreen tree or shrub 3 to 6 m high, with bright green ovate and deeply veined leaves which are 10 to 30 cm long. The fruit can grow in size up to 12 cm or more and has a lumpy surface covered by polygonal-shaped sections. The Polynesians utilized the whole plant in different combinations for herbal therapies. Noni fruit juice is in high demand in alternative medicine for different kinds of illness such as arthritis, diabetes, high blood pressure, muscle aches and pains, menstrual difficulties, headaches, heart disease, AIDS, cancers, gastric ulcers, sprains, mental depression, senility, poor digestion, atherosclerosis, blood vessel problems and drug addiction. A number of major components have been identified in the Noni plant such as scopoletin, octoanoic acid, potassium, vitamin C, terpenoids, alkaloids, anthraquinones (such as nordamnacanthal, morindone, rubiadin, and rubiadin-1-methyl ether, anthraquinone glycoside), b-sitosterol, carotene, vitamin A, flavone glycosides, linoleic acid, alizarin, amino acids, acubin, L-asperuloside, caproic acid, caprylic acid, ursolic acid, rutin and a putative proxeronine[9].

The present work is focused on physicochemical, preliminary phytochemical analysis, and antimicrobial activity of *M. citrifolia* fruits at different maturity stages (immature, midmature, mature) using various solvents.

MATERIALS AND METHODS

Collection of fruits

Different maturity stages (immature, midmature and mature) of *M. citrifolia* fruits were procured from World Noni Research Foundation (WNRF), Chennai, Tamil Nadu, India.

Physicochemical analysis

The physicochemical parameters such as total ash, water soluble ash, acid insoluble ash, loss on drying and water, alcohol, methanol, ethyl acetate, and chloroform soluble extractive values were determined using a standard procedure[10].

Preparation of fruit extracts

M. citrifolia fruits at maturity stages were washed with tap water followed by washing with distilled water. The fruits were peeled and the core was cut into small pieces and kept for shade drying and the dried fruit was then finely powdered using a mixer[11].

The powdered fruit material (15g) was extracted with 100ml of water, ethanol, methanol, ethyl acetate and chloroform separately. The contents were kept as such in room temperature for 48h with constant stirring at regular intervals. After the incubation period, the contents were filtered through Whatmann No.1 filter paper. Then filtrates were vacuum dried using rotary evaporator and concentrates were stored at 4°C. The residues were redissolved with the appropriate solvents from which they were prepared and used for further studies[12].

Preliminary phytochemical analysis

Qualitative phytochemical analyses were performed in filtrates of *M. citrifolia* fruits at different maturity stages were carried out to determine the presence of phytochemicals like carbohydrate, protein, tannin, flavonoid, saponin, steroids, alkaloids and glycosides as described by standard procedure[13, 14].

Test organisms

The microbial cultures such as *Escherichia coli* MTCC-433, *Klebsiella pneumoniae* MTCC-432, *Salmonella typhi* MTCC-733, *Aspergillus niger* MTCC-10180 and *Aspergillus flavus* MTCC-9064 were procured from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India.

Preparation of inoculum

The microorganisms were inoculated into nutrient broth and rose bengal broth for bioassay and incubated for 24 and 48 h at 37°C. The turbidity of the medium indicates the growth of organisms[15].

Antimicrobial studies

The agar well diffusion method was employed for the determination of antimicrobial activity of the extracts[16]. Lawn culture of *E. coli*, *K. pneumonia* and *S. typhi* were spread on nutrient agar and *A. niger* & *A. flavus* spread on rose bengal agar using sterile cotton swabs. The wells (6mm in diameter) were cut from the agar plates using a cork borer. 30µl of the extracts (7mg/ml) were poured into the well using a sterile micropipette. The plates were incubated at 37±2°C for 24 hours for bacterial activity and 48 hours for fungal activity. The plates were observed for the zone formation around the wells. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the well diameter.

RESULTS AND DISCUSSION

For the purpose of quality control, assessment of purity and identification of any sample, standardization is much essential[17]. The standardization of a crude drug is an integral part for establishing its correct identity[18]. Standardization including physicochemical evaluation is meant for identification, authentication and detection of adulteration and also compilation of quality control of crude drugs. The physical constant evaluation of the drug is an important parameter in detecting improper handling of drugs[19].

In the present study, physicochemical evaluation such as total ash, water soluble ash, acid insoluble ash, loss on drying, and extractive values were determined (Table 1 and Fig. 1). Ash value which is simply represents inorganic components naturally occurring in crude drug and also various impurities like carbonate, oxalate, and silicate[20]. The ash value was determined by three different methods, which are, total ash, water soluble ash, and acid insoluble ash. The total ash is employed to measure the total amount of material remaining after ignition. Acid insoluble ash is a part of total ash and measures the amount of silica present. Water soluble ash is the water soluble portion of total ash[21]. In our study, the total ash value of dried fruits of *M. citrifolia* was found to be in a range of 2%-4% while, water soluble was found in range between 1.5% and 2.5% and acid insoluble ash in range from 3% to 7.5%. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast, or fungi during storage, as the general requirement for moisture content in crude drug is not more than 14% w/w [22]. In our study the moisture content was found to be in a range between 3.5% and 6.5%. Extractive values are primarily useful for the determination of exhausted and adulterated drugs. It is also useful to evaluate the chemical constituents present in crude drug and helps in determination of specific constituents soluble in particular solvents[19, 20]. In the present study, the maximum extractive value was observed in aqueous extract of midmature *M. citrifolia* fruits.

Table 1: Physicochemical parameters of *M. citrifolia* fruits at different maturity stages

Parameters (% w/w)	IM	MM	M
Total ash	4.0	2.0	2.5
Water soluble ash	2.0	2.5	1.5
Acid insoluble ash	7.5	3.0	6.5
Moisture content (% dry weight basis)	3.5	6.5	4.0

IM = immature, MM = midmature, M = mature

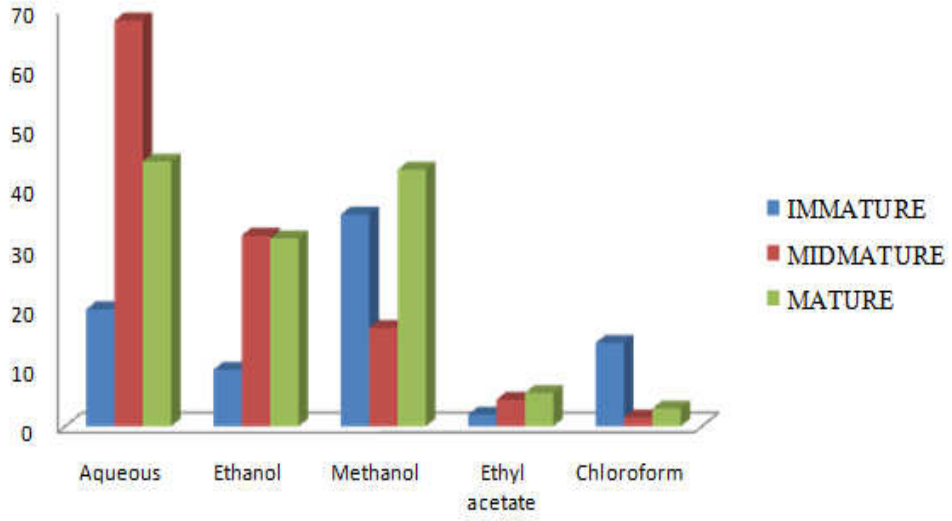

Fig. 1: Extractive values of *M. citrifolia* fruits at different maturity stages using various solvents

The medicinal plants are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, steroids, tannins and saponins. They are of great medicinal value and have been extensively used in drug and pharmaceutical industry. Recently the numbers of studies have been reporting the phytochemical study of medicinal plants. In the present investigation, different maturity stages of *M. citrifolia* fruits were investigated for the presence of various secondary metabolites using different solvents (Table 2). Phytochemical analysis of the aqueous extract showed the presence of carbohydrate, protein, alkaloids, saponin, glycosides, tannins, flavonoids and steroids. Ethanol extracts showed the presence of all the tested metabolies except saponins. Proteins were absent in the methanol extract. Alkaloids and saponins are not identified in ethyl acetate and choloroform extract.

Medicinal value of plants depends on the bioactive phytocomponents in plants associated to antibacterial activities. Hence, there is a need to focus on the traditional medicines which can serve as novel therapeutic agents [23]. The increased frequency of resistance to commonly used antibiotics leads to the search for new effective and easily affordable drugs in the management of infectious diseases[12]. In the present study, the antimicrobial activity was investigated in various maturity stages of *M. citrifolia* (Figs. 2 & 3). Aqueous extract of mature *M. citrifolia* showed positive result (5mm) against *E.coli,* whereas immature and midmature extracts not showed any inhibition. *K. pneumoniae* was susceptible to all the extracts of mature *M. citrifolia*. Aqueous extract showed 11mm inhibition zone to this organism, whereas immature aqueous extracts showed slight inhibition (5mm). *S.*

typhi was slightly inhibited by immature (8mm) and mature (7mm) by aqueous extract. Mature ethanol extracts showed moderate inhibition against *E.coli* (11mm). *K.pneumoniae* was inhibited by the mature ethanol extract. The zone formation was found to be 21mm to this organism, whereas immature extract showed 6mm and midmature extract showed 12mm zone of inhibition. *S. typhi* was strongly inhibited by mature ethanol extract (17mm), likewise mature ethanol extract showed positive result to *A. niger* (15mm) and *A.flavus* (18mm). Methanol extract of mature *M. citrifolia* shows 13mm inhibition zone against *E.coli*. *K. pneumoniae* was susceptible to all the three extracts of methanol. The inhibitory zone to this organism was 12mm, 13mm and 19mm respectively for immature, midmature and mature extract. *S. typhi* was strongly inhibited by immature (17mm) and mature (18mm) methanol extract. *A. niger* (18mm) and *A.flavus* (19mm) was inhibited by mature methanol extract. *E.coli* was susceptible to mature ethyl acetate extract (11mm), whereas it was slightly inhibited by midmature (4mm) and mature (5mm) of chloroform extract. Mature ethyl acetate (11mm) and chloroform extracts (15mm) showed a positive result against *K. pneumoniae*. *A. niger* (11mm) and *A. flavus* (13mm) was inhibited by mature ethyl acetate and chloroform extracts respectively.

Table 2: Phytochemical analysis of extracts of *M. citrifolia* fruits at different maturity stages

Metabolites	Aqueous			Ethanol			Methanol			Ethyl acetate			Chloroform		
	IM	MM	M	IM	MM	M	IM	MM	M	IM	MM	M	IM	MM	M
Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Proteins	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavonoid	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
Saponin	+	+	+	-	-	-	+	+	+	-	-	-	-	-	-
Steroids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Present, - = Absent, IM = immature, MM = midmature, M = mature

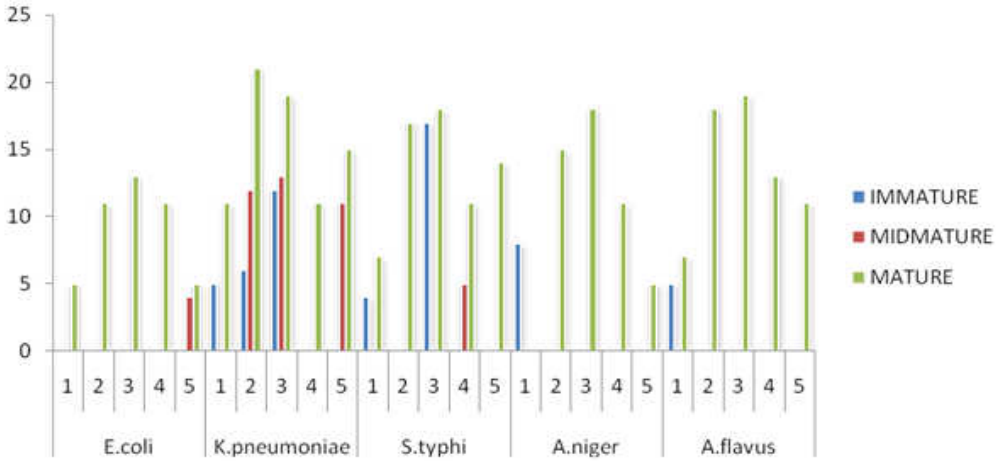

Fig. 2: Antimicrobial activity of extracts of *M. citrifolia* fruits at different maturity stages (1-aqueous; 2-ethanol; 3-methanol; 4-ethyl acetate; 5-chloroform)

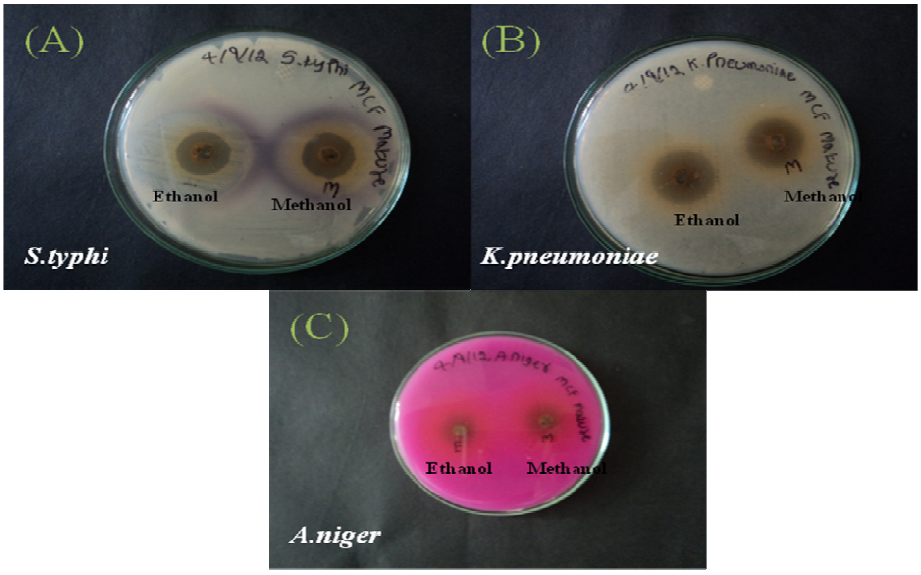

Fig. 3: Antimicrobial activity of extracts of *M. citrifolia* fruits at different maturity stages. (A) Antibacterial activity against *S. typhi*; (B) Antibacterial activity against *K. pneumoniae*; (C) Antifungal activity against *A. niger*

CONCLUSION

In conclusion, the results of physicochemical parameters in the present study would be the evidence for the identification and purity of the *M. citrifolia* fruits. The presence of various secondary metabolites such as alkaloids, flavonoids, saponins and steroids in different maturity stages of fruit providing the promising effect on antimicrobial activity against tested pathogenic organisms. These data will be adding the scientific evidence for antimicrobial efficacy of *M. citrifolia* fruits which could be useful for the development of safe and effective novel therapeutic agents.

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