Journal of Research in Microbiology

E-ISSN: 2709-944X
P-ISSN: 2709-9431
JRM 2021; 2(1): 13-21

www.microbiojournal.com
Received: 19-01-2021
Accepted: 11-03-2021

JP Nikkitha
[a] Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India
[2] Department of Botany, Thiruvalluvar Government Arts College, Rasipuram, Namakkal (Dt), Tamil Nadu, India

P Suresh
Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

S Kameshwaran
Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

M Rekha
Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

G Mahendra Perumal
Department of Botany, V.H.N.S.N. College, Virudhunagar, Tamil Nadu, India

V Shanmugaiah
Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

Correspondence
V Shanmugaiah
Department of Microbial Technology, School of Biological Sciences, Madurai Kamaraj University, Madurai, Tamil Nadu, India

Bioactive metabolites from ethyl acetate extract of leaves of *Melia dubia* L., against human and plant microbial pathogens

JP Nikkitha, P Suresh, S Kameshwaran, M Rekha, G Mahendra Perumal and V Shanmugaiah

Abstract
Antimicrobial metabolites are screening from medicinal plants is a promising technique to combat the growing issue of drug-resistant infections in humans and plants. In the present study, antimicrobial activity of *Melia dubia* L., was tested against human and plant pathogenic microbes by well diffusion method. Among three solvent system, ethyl acetate extract of *M. dubia* was showed significant activity towards human and plant pathogens such as *Escherichia coli* MTCC443, *Salmonella typhi* MTCC733, *Bacillus cereus* MTCC 430, *Staphylococcus epidermidis* MTCC 10623, *Klebseilla* sp. MTCC 3384, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Sclerotium rolfsii* and *Fusarium oxysporum* with different level of zone of inhibition (ZOI) from 2 mm to 15 mm and 5 mm to 27 compared to control. The maximum quantity of phytochemical constituents like tannins, total phenol, flavonoids, saponins and alkaloids were obtained from ethyl acetate extract of *M. dubia* in comparison with other two solvent system such as acetone and petroleum ether. From ethyl acetate extracts of *M. dubia*, four spots of biomolecules with Rf values of 0.46, 0.41, 0.32 and 0.18 were detected on thin layer chromatography (TLC). Bioactive compounds are now undergoing purification and characterisation from *M. dubia*.

Keywords: *M. dubia* L, phytochemical constituents, bioactive compounds. TLC and antimicrobial activity

Introduction
The herb *Melia dubia* L., also known as Hill Neem, Malai Vembu, Munnattikaraka, and it would be used as an andithelmentic, as well as for gastrointestinal and colic diseases (Saini *et al*., 2007) [29]. Chemical pesticides', antibiotics and fungicides are cause severe threat to the human being and affect the plant health. Problem of drug-resistant microbes, have spurred to scientists to explore for more effective and environmentally benign alternatives in recent years (Aktar *et al*., 2009; Akacha *et al*., 2016) [2, 1]. In truth, microbial resistance is becoming a bigger issue, and the future of antimicrobial drug use is still up in the air. Furthermore, due to the many adverse effects of anti-inflammatory medicines, treating chronic inflammatory illnesses is challenging and impacts for humans (Nascimento *et al*., 2000; Li *et al*., 2003) [21, 16]. Infectious disease is the major cause of death in underdeveloped countries, accounting for over half of all deaths (Murtaza *et al*., 2015) [19]. Plants are a major source of raw materials for medicines, which are used to treat a wide range of human ailments. Because of their compatibility with our biological system, natural-source medications have captivated the interest of modern civilization (Amalraj, 1983; Paritala *et al*., 2014) [3, 23]. Scientific study on medicinal plants is centered on the discovery of active principles in plants, as well as a scientific examination of remedies that leads to product standardization and quality control to assure product safety. After passing specific tests, they may be approved for use in primary health care. In this case, previous research initiatives may have resulted in the development of new medications. (Farnsworth, 1988; Paritala *et al*., 2014; Goswami *et al*., 2020) [8, 23, 10].
Antibiotic-resistant bacteria are becoming more common, and synthetic treatments may have negative side effects (Priya *et al*., 2020) [25]. In order to find novel sources of plant medications, several plants have been investigated for a wide range of biological activity in various research organizations. Because, they have so much therapeutic potential (Sandhya *et al*., 2006; Paritala *et al*., 2014), plant-based antibacterial activity represents a significant

untapped source of pharmaceuticals (Sandhya *et al*., 2006; Paritala *et al*., 2014) [30, 23]. Furthermore, as people become more aware of the benefits of natural plant-based medications, which are nontoxic, have no side effects, and are widely available at a reasonable cost, demand for herbal treatments is rising around the world. Herbal medicines are currently used as a primary health care system by more than 80% of the world's population (Gopal *et al*., 2015) [9]. Traditional medicine plants include a wide range of chemicals that can be utilized to treat both chronic and infectious disorders. As a result, there is an increasing need to evaluate medicinal plants' antibacterial and antifungal capabilities in order to use them in green medicine, as they are regarded to be safer and less expensive than conventional medications (Rajkumar and Malathi, 2015; Canli *et al*., 2015; Murtaza *et al*., 2015) [27, 5, 19]. Among the various families with recognized pharmacological properties, the Meliaceae family deserves special for the control microbial pathogens and other neurogenic disorder diseases.

M. dubia is a species native of southern Asia (India, Pakistan and Iran), but it has also been introduced to South Africa, America (Bermuda, Brazil, and Argentina), middle East, southeast Asia-Pacific Islands, Australia, and southern europe (Ram *et al*., 2007; Shah *et al*., 2016) [28, 32]. *M. dubia* is a huge deciduous tree native to India that has recently become one of the most extensively planted tree species in the southern part of the country. All components of the *M. dubia* plant are used as traditional herbal medicines for anthelmintics, malaria, leprosy treatment, asthma, eczema, fevers, acariasis, cholelithiasis and joints pain (Govindachari, 1992; Dinesh *et al*., 2020) [11, 7]. The antibacterial, antiviral, antifungal, antineoplastic, antidiabetic, antihelmintic, and antileprosy capabilities of various portions of the *M. dubia* with diverse extracts have been exploited in pharmaceutical importance (Pettit *et al*., 2002; Nagalakshmi *et al*., 2003; Susheela *et al*., 2008; Shah *et al*., 2016) [24, 20, 36, 32] Phytochemical screening experiments revealed that *Melia dubia* produces a wide range of biochemical compounds, including alkaloids, saponins, glycosides, oleoresins, resins, sesquiterpene lactones and oil making it a biochemical abundant bioresource (Leela *et al*., 2016; Jeyaleela *et al*., 2017) [15, 13]. Many researchers have focused their attention on phytochemicals from medicinal plants and their ability to stop human diseases (Senguttuvan *et al*., 2014) [31]. Antimicrobial and antifungal characteristics of *M. dubia* leaves and bark extracts were evaluated against gram positive and gram-negative bacteria as well as human pathogenic fungi (Saini *et al*., 2007; Mudhafar *et al*., 2020) [29, 18]. However, researchers were indicated that *M. dubia* leaves contain a large number of bioactive molecules, hence purification, characterization, identification and their application towards medical fields need to be investigated for welfare of human kind. The present was carried with the following objectives

i) to investigate the antibacterial properties of *M. dubia* leaf extracts against human bacterial pathogens and plant pathogenic fungi,
ii) To study the phytochemical properties of *M. dubia*,
iii) Detection of antimicrobial molecules on TLC.

Materials and Methods

Collection of plant material

M. dubia were collected in Tamil Nadu's Kolli hills area, Namakkal district, Tamil Nadu. Plant leaves were completely washed with tap water to remove soil particles and other adherent detritus, then sun shade dried for 10-14 days before being crushed finely. Powdered ingredients were kept in a zip-lock plastic bag until they were needed.

Extraction of *M. dubia* leaves with different solvents

10g of dry plant powder was soaked in 200ml of ethyl acetate, petroleum ether and acetone solvents for 24 hours before being stored at room temperature. Filtered samples were used for phytochemical analysis and antibacterial activity testing. The filtrate was collected in a separate container after being filtered through a Whatman no. 1 filter paper in a funnel. The filtrate was evaporated to dryness, yielding a crude extract of *M. dubia* was used for further research. (Gopal *et al*., 2012) [9].

Phytochemical's analysis

Estimation of Alkaloids

2 g of M. dubia leaf dry powder was mixed with 100 mL of 20% acetic acid in 500mL beaker covered with aluminium boil and kept at room temperature for 12 h. The volume of this solution-containing combination was lowered to one-quarter using a water bath. Ammonium hydroxide was added drop by drop until the precipitation was complete. The entire solution was allowed to settle before the precipitate was filtered and weighed. (Senguttuvan *et al*., 2014) [31]. The total alkaloid content was computed as a percentage of the total alkaloid content: Percentage of total alkaloids (%) = Weight of residue X 100/Weight of sample taken

Estimation Total flavonoids content

The total flavonoids content was calculated using a modified version of the approach given by Zhishen *et al*., 1999 [38]. A total of 1 ml of plant extracts were diluted with 200mL of distilled water before being added to a 150 µl solution of 5% sodium nitrite. After 5 min of incubation, a 10% aluminium chloride (150 µl) solution was added and left to stand for 6 min. Then 2 mL of sodium hydroxide (4%) solution was added and distilled water was added to make up to 5 mL. The liquid was thoroughly mixed before being left at room temperature for 15 min. The presence of flavonoids was detected by the appearance of pink color, which was measured at 510 nm. The total flavonoids content was expressed as quercetin equivalent mg QE/g of leaf extract on a dry weight basis.

Estimation of total phenolics content

Total phenol content from *M. dubia* was quantified by the modified method of Sidduraju and Becker, 2003 [34] using the Folin-Ciocalteau reagent. Separately, 20 µl of leaf extracts were collected and mixed with 1 ml of distilled water. 500 µl of diluted Folins-phenol reagent (1N) was added, along with 2.5 ml of sodium carbonate (20%). For the development of color, the mixture was vigorously mixed and incubated in the dark for 40 min. The absorbance was measured at 725 nm after incubation. Using the standard curve, the total phenolics content in the plant extracts was quantified as mg of gallic acid equivalent (mg GAE/g extract).

Estimation of total saponins content

The net saponin content was estimated using a slightly

modified approach based on the vanillin-sulphuric acid colorimetric reaction developed by Makkar *et al*. (2007) [17]. A total of 50 µl of plant extract were mixed with 250 µl of distilled water. A total of 250 µl of vanillin reagent (800 mg vanillin in 10 mL 99.5 percent ethanol) were added. 2.5 mL of sulphuric acid (72%) was added and thoroughly mixed. This solution was held at 60°C for 10 minutes in a water bath. It was chilled in ice cold water for 10 minutes before the absorbance was measured at 544 nm. The results were calculated using a standard curve and represented as diosgenin equivalents (mg DE/g extract).

Estimation of tannins content

Tannin content was estimated from *M.dubia* by the modified method of Siddhuraj and Manian, 2007 [35]. In a separate test tube, 500 µl of the extracts were treated with 100 mg of polyvinyl polypyrrolidone (PVP) and 500 µl of distilled water. This solution was incubated for 4 h at 4 °C. The sample was centrifuged for 5 min at 5000 rpm, and 20 µl of the supernatant was collected. There are no tannins in this supernatant, simply basic phenolics (the tannins would have been precipitated along with the PVP). The quantity of free phenolics in the supernatant was measured at 725 nm and represented as a percentage of dry content. The extract's tannin concentration was determined as follows:

Tannins (mg GAE/g extract) = Total phenolics (mg GAE/g extract)-Free phenolics (mg GAE/g extract).

Antimicrobial activity of ethyl acetate *M.dubia* leaf against human pathogenic bacteria and plant pathogenic fungi

Agar well diffusion was used to investigate the antibacterial activity of crude extracts of *M.dubia* against human pathogens. The human pathogenic bacteria such as *Escherichia coli* MTCC443, *Salmonella typhi* MTCC733, *Bacillus cereus* MTCC 430, *Staphylococcus epidermidis* MTCC 10623 and *Klebseilla* sp. MTCC 3384 were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India was used in this investigation. The pure culture of bacteria was grown in Luria Bertani broth (LB) for 24 h at 37°C. The overnight grown bacterial culture and their turbidity was adjusted to 1 O.D at 600NM (approximately 1X 10^8 CFU/ml). 100 µl aliquots of bacterial inoculums were spread over the surface of Muller-Hinton agar medium (MHA). Using sterile buds. After that agar wells were made using sterile cork borer. Each well was added with different concentrations of crude extracts of *A. indicum* at 25 to 200 µg/ml. Negative and positive controls were sterile distilled water and tetracycline [38]. At room temperature, the plates were incubated for 24 hours. After incubation, the outcome was detected, and the zone of inhibition was measured in comparison to the control. The result was noticed after incubation, and the zone of inhibition was assessed in comparison to the control.

The antifungal activity of *M.dubia* leaf crude extract was performed by well diffusion method[39].The crude extracts were diluted in ethyl acetate and then injected at doses ranging from 25 to 200 µg/ml into wells on a Potato Dextrose Agar (PDA) plate. In the centre of PDA plates, fungal mycelial discs (9mm) from *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, and *Fusarium oxysporum* were introduced and cultured for three days at 28±2°C. The mycelial growth of the fungus was measured after three days and compared to the control. In all of the experiments, three replications were used.

Thin layer chromatography (TLC)

TLC was performed on a pre-coated silica gel thin layer chromatogram sheet (TLC Silica gel 60 F254, 20X20 cm, Merck).The ethyl acetate extract was spotted from the edge of the sheet. The chromatogram is developed with a mixture of a suitable solvent system using Hexane: Ethyl acetate (3.5:1.5) and dried at room temperature (Jeyaleela *et al*., 2017) [13]. The spots were photographed in a UV chamber. Each spot's Rf values were recorded.

Results and Discussion

Phytochemical analysis

Among the three solvent systems, ethyl acetate leaf extracts of M. dubia contained the most phytochemicals such as alkaloids, flavonoids, total phenol, saponin, and tannin when compared to acetone and petroleum ether extracts. Ethyl acetate leaf extract of *M. dubia* in ethyl acetate had the highest concentration of saponins (11.18mg AE/100 g extract**)** and flavonoids (8.5 mg GAE/100 g extract) content was observed compared to other phytochemicals like alkaloid (0.69mg/g), tannins (0.71 mg GAE/g extract) and phenol (0.51mg GAE/100 g extract) (Fig. 1. A-E). According to the results of the phytochemical screening, alkaloids, tannins, phenolic compounds, saponins and flavonoids were found in all fractions except hexane, as indicated in table 1. According to our findings, the presence of alkaloids, tannins, terpenoids, flavanoids and phenolics in methanolic extracts of *M. dubia* was discovered (Dinesh *et al*., 2020) [7]. This species is believed to have a wide variety of medical applications since these secondary metabolites are said to have a wide range of biological and therapeutic applications (Proestos *et al*., 2014; Senguttuvan *et al*., 2014) [26, 31]. When ethyl acetate extract was compared to other solvents, considerable amounts of phytochemicals were produced. Whereas, according to Senguttuvan *et al*., 2014 [31], the extraction yield of phytochemicals from H. *radicata* was calculated for petroleum ether, chloroform, ethyl acetate, methanol, and water extracts, with methanol extract having the highest percentage yield of phytochemicals.

Alkaloids, tannins and saponins are powerful metabolites that effectively fight a wide range of human and plant pathogens. However, in concurrence with our study, some other recent report revealed that, more yield of phytochemicals was obtained from ethanolic and ethyl acetae fractions of *M. dubia* leaves (Leela *et al*., 2016) [15]. Antimicrobial, antioxidant, antidiabetic and anticancer effects are hypothesized to be attributed to phenols and flavonoids. Phytochemicals are play an important role in protecting against damage to membrane functions (Dinesh *et al*., 2020) [7]. In general, phytochemicals are scientifically used to control most of the multidrug-resistant bacteria and plant pathogenic microbes due to the number of phytochemicals obtained from medicinal plants. Phytochemicals are able to release hydroxy radicals and that are highly reactive free radicals that arise in medicinal plants and other biological systems. They were caused significant damage to nearly every molecule identified in living cells of pathogens. These radicals have the ability to link nucleotides in DNA, causing strand breaking, which contributes to carcinogenesis, mutation, and cytotoxicity. They are also one of the fastest initiators of the lipid peroxidation process (Dinesh *et al*, 2020) [7].

Effect of *M. dubia* extracts against antibacterial and antifungal activity

The antimicrobial activity of *M. dubia* leaves of various solvent extract against different human pathogens both gram-positive and gram-negative bacteria. Among three different solvent extracts the significant activity was observed in ethyl acetate followed by acetone and petroleum ether. The ethyl acetate extracts of *M. dubia* was showed maximum activity against *S. paratyphi* and *E.coli* (8 and 7 mm) respectively (Karthikeyan *et al*., 2014) [14]. Chanthuru *et al*. (2014) [6] reported that *Melia dubia* leaf extract ethyl acetate extract of *M. dubia* leaf extract at the concentration of 60 µl showed prominent zone of inhibition activity against all the bacterial pathogens like *Salmonella typhi*, *Escherichia coli*, *S. paratyphi*, *Staphylococcus aureus* and *Klebsilla peneumonia* (20, 28, 18, 22 and 26 mm respectively). In concurrence with previous report, The current study showed that *M. dubia* ethyl acetate leaf extracts showed significant activity against five different human pathogenic bacteria such as *S. epidermidis*, *S. typhi* and *Klebseilla* sp. at 25 µg/ml. However, the complete inhibition of *B. cereus* and *E. coli* at 100 µg/ml was observed with different level inhibition zone (ZOI) from 10 mm and 9 mm respectively (Table 2; Fig. 2A-E). The acetone extracts of *M. dubia* demonstrated activity against only *S. epidermidis* at 200 µg/ml with 7 mm ZOI compared to control (Table 4). Whereas, petroleum ether leaves extract of *M. dubia* but did not showed any activity towards human pathogenic bacteria (Table 3). The present study is highly concurrence with previous study, the ethyl acetate extract of *M. dubia* showed maximum activity against human pathogens (Chanthuru *et al*., 2014) [6]. *M. dubia* leaves methanolic extracts were showed prominent antibacterial activity towards *E. coli* and *S. mutans* (Dinesh *et al*, 2020) [7]. As a result, our research showed that this plant has the potential to be a useful therapeutic plant for humans.

The ethyl acetate leaf extract of *M.dubia* demonstrated significant antifungal activity against *R. solani*, *M. phaseolina*, *S. rolfsii* and *F. oxysporum*, all of which can cause fungal infection in a variety of cereals and legumes. The maximum antifungal activity by leaf extract of *M.dubia* was observed towards *S. rolfsii* at 125 µg/ml with complete inhibition compared to control. Whereas, the minimum antifungal activity against *R. solani*, *M. phaseolina* and *F. oxysporum* with 23, 20 and 5 mm of ZOI at 125 µg/ml compared to control (Table 5; Fig. 3A-D). The methanol and ethanol *M.dubia* leaves extracts were showed remarkable antifungal activity against four clinically significant fungal strains, including *Candida albicans*, *Aspergillus niger*, *A. flavus* and *A. fumigatus* compared to control (Leela *et al*., 2016) [15].

The antifungal activities of methanolic leaf extracts of *L. camara* considerably inhibited the growth of *Aspergillus fumigatus* and *Aspergillus flavus* [56], these findings are highly coinciding with our results. The ethanolic extracts of *A. indicum* were significantly control the growth of human fungal pathogens such as *Candida albicans* and *Aspergillus niger* than the aqueous extracts of *A. indicum* compared to control [57]. Since phytochemical extracts and their biochemical components play a direct role towards fungal biochemical development, through which fungal growth must be halted. *L. inermis* extracts inhibited the development of catalase enzyme in *A. niger* and *F. oxysporum.* In fungi, because catalase is very much needed for the conversion of H_2O_2 to oxygen and water. If catalase not produced, H_2O_2 can build up in large amounts and become toxic to the fungal cell [58]. The extracts from *Acacia arabica* and *Casuarina equisetifolia* was inhibited, the cellulolytic enzyme in *Pythium aphanidermatum*. The inability and inhibition of cellulase enzyme by action of inhibitor compound present in the plant extracts. Gupta and Bilgrami [59]. Since the fungal cell wall of Oomyctes is made up of cellulase, if the enzyme's function is inactivated, Oomyctes will be unable to survive and cause any diseases in plant system. As a result, phytochemicals are thought to be more effective components in the management of pathogens caused human and plant diseases.

Exposure of antimicrobial compounds from leaves of *M. dubia* on TLC

The ethyl acetate extract of leaves of *M. dubia* yielded a total of four biomolecules on TLC with different Rf values such as 0.18, 0.32, 0.41 and 0.46 (Fig. 4). Antimicrobial compounds are identified using TLC from various solvent extracts. On TLC, many functional groups of compounds were discovered in plant extracts, each with a different Rf value. (Gupta *et al*., 2013) [12]. Many components in leaf extracts resolved on TLC plates with obvious bactericidal zones, indicating that antibacterial chemicals are polar in nature. The bulk of the phytochemicals were found in ethyl acetate, methanol, or ethanol extracts. As a result, TLC detected several phytochemicals such as flavonoids, saponins, tannins, alkaloids, and phenols with varying Rf values.

Conclusion

The *M. dubia* plant could be employed as a biomedical and biocontrol agent to combat human and plant pathogen-caused disorders.

Tables

Table 1: Quantification of phytochemical analysis of leaves of *Melia dubia* with different solvent system

S. No.	Phytochemicals	Ethyl acetate	Petroleum ether	Acetone
1	Tannins	0.71±0.06	0.44±0.04	0.53±0.07
2	Total Phenol	0.51±0.03	0.25±0.03	0.17±0.02
3	Flavanoids	8.5±0.39	5.99±0.29	4.63±0.34
4	Saponins	11.18±0.3	6.37±0.29	5.5±0.39
5	Alkaloids	0.69±0.47		

The values are triplecates with standard deviation. Tannins (mg RE/100 g extract); Total Phenol (mg GAE/100 g extract); Flavanoids (mg GAE/100 g extract); Saponins (mg AE/100 g extract); Alkaloids (mg /100 g of sample)

Table 2: Evaluation of antibacterial activity by leaves of *M. dubia* Ethyl acetate extract against human pathogens

S. No	Human pathogens	Conc. µg/ml / Zone of Inhibition (mm)							
		25	50	75	100	125	150	175	200
1	*E. coli*	4	4	7	9	++	++	++	++
2	*S. epidermidis*	++	++	++	++	++	++	++	++
3	*Klebseilla*	++	++	++	++	++	++	++	++

4	*B. cereus*	2	4	5	7	10	10	11	15
5	*S. typhi*	++	++	++	++	++	++	++	++

++: Complete inhibition

Table 3: Evaluation of antibacterial activity by leaves of *M. dubia* petroleum ether extract against human pathogens

S. No	Human pathogens	Conc. µg/ml / Zone of Inhibition (mm)							
		25	50	75	100	125	150	175	200
1	*E. coli*	-	-	-	-	-	-	-	-
2	*S. epidermidis*	-	-	-	-	-	-	-	-
3	*Klebseilla*	-	-	-	-	-	-	-	-
4	*B. cereus*	-	-	-	-	-	-	-	-
5	*S. typhi*	-	-	-	-	-	-	-	-

- : No inhibition

Table 4: Evaluation of antibacterial activity by leaves of *M. dubia* acetone extract against human pathogens

S. No	Human pathogens	Conc. µg/ml / Zone of Inhibition (mm)							
		25	50	75	100	125	150	175	200
1	*E. coli*	-	-	-	-	-	-	-	-
2	*S. epidermidis*	-	-	-	-	2	4	5	7
3	*Klebseilla*	-	-	-	-	-	-	-	-
4	*B. cereus*	-	-	-	-	-	-	-	-
5	*S. typhi*	-	-	-	-	-	-	-	-

- : No inhibition

Table 5: Evaluation of antibacterial activity by leaves of *M. dubia* ethyl acetate extract against human pathogens

S. No	Fungal Plant Pathogens	Conc. µg/ml / Zone of Inhibition (mm)							
		25	50	75	100	125	150	175	200
1	*R. solani*	10	12	13	14	23	24	25	25
2	*M. phaseolina*	12	13	13	15	20	25	26	27
3	*S. rolfsii*	13	15	15	16	++	++	++	++
4	*F. oxysporum*	-	-	-	-	5	5	7	8

++ : Complete inhibition; - : No inhibition

Figures

Fig 1 Phytochemicals analysis of *M. dubia* in different solvent extracts

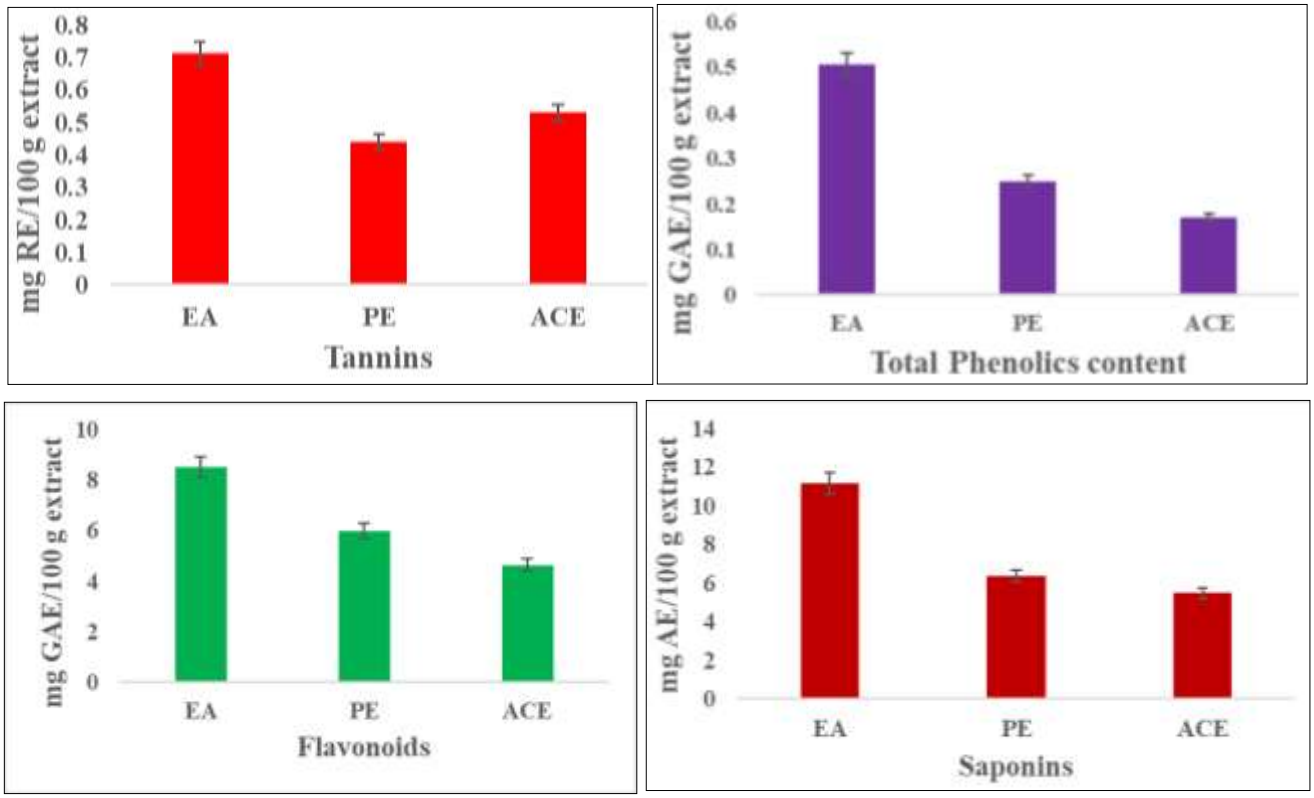

Fig 1: Phytochemicals analysis of *M. dubia* in different solvent extracts. A – Tanins, B – Total phenolics content, C – Flavonoids and D – Saponins.

Fig 2 Effect of *M. dubia* ethyl acetate extracts against human pathogen

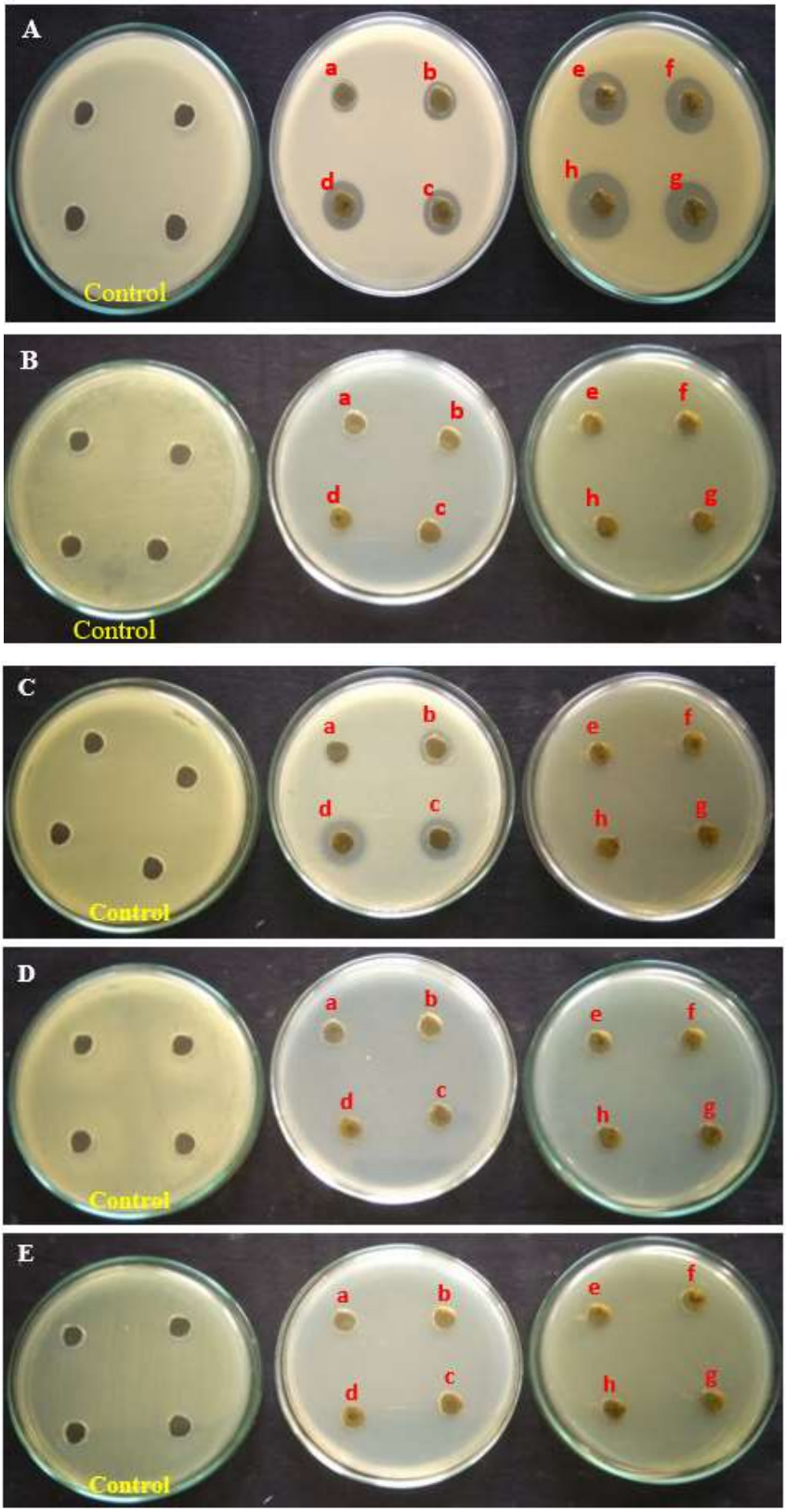

Fig 2: Effect of *M. dubia* ethyl acetate extracts against human pathogen. A - *B. cereus*, B - *S. epidermidis*, C - *E. coli*, D - *S. typhi*, and E - *Klebseilla* sp. a: 25, b: 50, c: 75: d: 100: e: 125: f: 150, g: 175 and h: 200 µg/ml which represent the concentration of crude metabolites.

Fig 3 Effect of *M. dubia* ethyl acetate extracts against phyto fungal pathogens

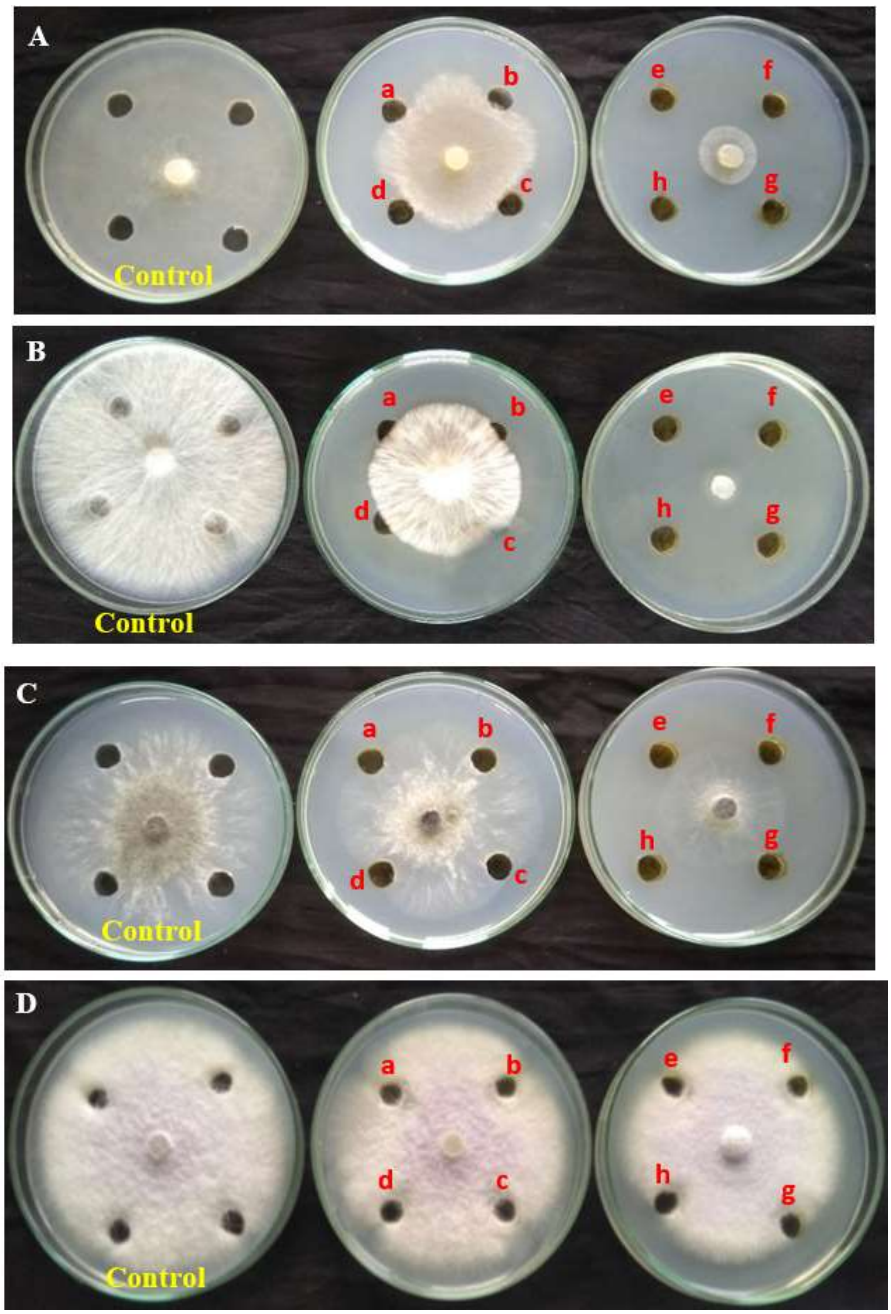

Fig 3: Effect of *M. dubia* ethyl acetate extracts against phyto fungal pathogens. A - *R. solani*, B - *S. rolfsii*, C - *M. phaseolina*, and D - *F. oxysporum*. a: 25, b: 50, c: 75: d: 100: e: 125: f: 150, g: 175 and h: 200 µg/ml which represent the concentration of crude metabolites.

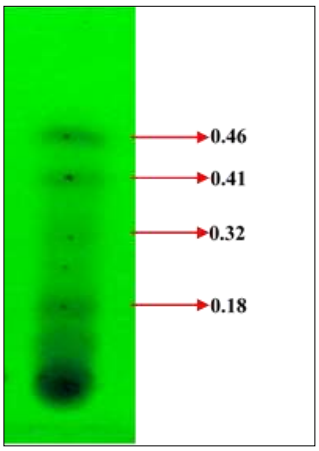

Fig 4: Detection of metabolites on TLC from leaves of *M. dubia* ethyl acetate extracts

References

1. Akacha M, Lahbib K, Daami-Remadi M, Boughanmi NG. Antibacterial, antifungal and anti-inflammatory activities of *Melia azedarach* ethanolic leaf extract. Bangladesh Journal of Pharmacology 2016;11(3):666-674.
2. Aktar W, Sengupta D, Chowdhury A. Impact of pesticides use in agriculture: their benefits and hazards. Interdisciplinary toxicology 2009;2(1):1-2.
3. Amalraj VA. Secondary Plant Constituents. Sci. Rep., June issue, CSIR, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, Calcutta 1983.
4. Bardas GA, Veloukas T, Koutita O, Karaoglanidis GS. Multiple resistance of *Botrytis cinerea* from kiwifruit to SDHIs, QoIs and fungicides of other chemical groups. Pest management science 2010;66(9):967-973.
5. Canli K, Altuner EM, Akata I. Antimicrobial screening of *Mnium stellare*. Bangladesh Journal of Pharmacology 2015;10(2):321-25.
6. Chanthuru A, Prabhu MM, Aysha OS, Karthik R. Evaluation of leaf and root extracts of *Melia dubia* L. against larvae of Culex quinquefasciatus and five important human pathogens. Biosciences Biotechnology Research Asia 2014;11(1):207-10.
7. Dinesh B, Mahesh P, Shanthala M, Shalini V, Sindhu R, Viji KN, *et al*. Evaluation of the Phytochemical, Antioxidant and Anti-microbial Activities obtained from the Methanolic Leaf Extracts of *Melia dubia* Cav. Transactions on Science and Technology 2020;7(4):189-97.
8. Farnsworth NR. Screening plants for new medicines. Biodiversity 1988;15(3):81-99.
9. Gopal V, Prakash Yoganandam G, Manju P. A concise review on *Melia dubia* Cav. (Meliaceae). Euro J Environ *E.coli* 2015;2:57-60.
10. Goswami M, Bhagta S, Sharma D. *Melia dubia* and its Importance: A Review. International Journal of Economic Plants 2020;7(1):029-33.
11. Govindachari TR. Chemical and biological investigations on Azadirachta indica (the neem tree). Current Science 1992;63(3):117-122.
12. Gupta VK, Kaur C, Simlai A, Roy A. Antimicrobial activity of Pavetta indica leaves. Journal of Applied Pharmaceutical Science 2013;3(4):78.
13. Jeyaleela GD, Monisha SI, Vimala JR, Immaculate AA. Isolation of 2-chlorobenzimidazole from *Melia dubia* leaf extract and its structural characterisation. Mass spectrometry 2017;9:10.
14. Karthikeyan J, Nila KM, Thooyavan G, Vimalkumar E. Larvicidal and antibacterial efficacy of green synthesized silver nanoparticles using *Melia dubia*. Int J Pharm Pharm Sci 2014;6(7):395-9.
15. Leela GD, Monisha SI, Immaculate AA, Vimala JR. Studies on Phytochemical, Nutritional Analysis and Screening of *In vitro* Biological activities of *Melia dubia* Leaf Extract. International Journal of Scientific & Engineering Research 2016;7(8):56-68.
16. Li RW, Myers SP, Leach DN, Lin GD, Leach G. A cross-cultural study: anti-inflammatory activity of Australian and Chinese plants. Journal of ethnopharmacology. 2003;85(1):25-32.
17. Makkar HP, Siddhuraju P, Becker K. Methods in molecular biology: plant secondary metabolites. Plant Secondary Metabolites 2007;393:47-49.
18. Mudhafar M, Zainol I, Jaafar CN, Alsailawi HA, Majhool AA, Alsaady M. Phytochemical Screening and Characterization of *Melia dubia* Leaves Extract for Antimicrobial Activity against Escherichia coli and Staphylococcus aureus. Indian Journal of Ecology 2020;47(2):493-496.
19. Murtaza G, Mukhtar M, Sarfraz A. A review: Antifungal potentials of medicinal plants. Journal of Bioresource Management 2015;2(2):4.
20. Nagalakshmi MA, Thangadurai D, Pullaiah T. *In vitro* antimicrobial efficacy of leaf essential oils of *Chukrasia tabularis* Adr. Juss. and *Melia dubia* Cav. (Meliaceae). Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 2003;17(4):414-6.
21. Nascimento GG, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian journal of microbiology 2000;31(4):247-256.
22. Neycee MA, Nematzadeh GH, Dehestani A, Alavi M. Assessment of antifungal effects of shoot extracts in chinaberry (*Melia azedarach*) against 5 phytopathogenic fungi. Int J Agric Crop Sci 2012;4:474-477.
23. Paritala V, Chiruvella KK, Thammineni C, Ghanta RG, Mohammed A. Phytochemicals and antimicrobial potentials of mahogany family. Revista Brasileira de Farmacognosia 2015;25(1):61-83.
24. Pettit GR, Numata A, Iwamoto C, Morito H, Yamada T, Goswami A, *et al*. Antineoplastic Agents. 489. Isolation and Structures of Meliastatins 1− 5 and Related Euphane Triterpenes from the Tree Melia d ubia. Journal of natural products 2002;65(12):1886-1891.
25. Proestos C, Lytoudi K, Mavromelanidou OK, Zoumpoulakis P, Sinanoglou VJ. Antioxidant capacity of selected plant extracts and their essential oils. Antioxidants 2013;2(1):11-22.
26. Rajkumar K, Malathi R. Phytochemical investigation, GC-MS analysis and *In vitro* antimicrobial activity of *Coleus forskohlii*. Bangladesh Journal of Pharmacology 2015;10(4):924-930.
27. Ram B, Rathore TS, Bopanna BD. An efficient protocol for micropropagation and genetic stability analysis of *Melia dubia* Cav.-an important multipurpose tree. Int J Curr Microb App Sci 2014;3(7):533-544.
28. Saini V, Kinger HK, Middha A, Rathore GS. Study of anti-bacterial and anti-fungal activity of *Melia dubia* Leaves. Internet Journal of Plant Sci 2007;2:239-240.
29. Sandhya B, Thomas S, Isabel W, Shenbagarathai R. Ethnomedicinal plants used by the Valaiyan community of Piranmalai hills (reserved forest), Tamilnadu, India.-a pilot study. African Journal of Traditional, Complementary and Alternative Medicines 2006;3(1):101-114.
30. Senguttuvan J, Paulsamy S, Karthika K. Phytochemical analysis and evaluation of leaf and root parts of the medicinal herb, *Hypochaeris radicata* L. for *In vitro* antioxidant activities. Asian Pacific journal of tropical biomedicine 2014;4:S359-S367.
31. Shah SN, Wani TA, Ram B, Koul M, Awasthi P, Rajput DS, *et al*. An efficient protocol for *In vitro* organogenesis and antioxidant studies in *Melia dubia*

Cav. African Journal of Biotechnology 2016;15(19):768-775.
32. Shanmugaiah V, Mathivanan N, Varghese B. Purification, crystal structure and antimicrobial activity of phenazine-1-carboxamide produced by a growth-promoting biocontrol bacterium, Pseudomonas aeruginosa MML2212. Journal of Applied Microbiology 2010;108(2):703-711.
33. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. Journal of agricultural and food chemistry 2003;51(8):2144-2155.
34. Siddhuraju P, Manian S. The antioxidant activity and free radical-scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food Chemistry 2007;105(3):950-958.
35. Susheela T, Balaravi P, Theophilus J, Reddy TN, Reddy PU. Evaluation of hypoglycaemic and antidiabetic effect of *Melia dubia* CAV fruits in mice. Current science 2008, 1191-1195.
36. Valentina P, Ilango K, Kiruthiga B, Parimala MJ. Preliminary phytochemical analysis and biological screening of extracts of leaves of *Melia dubia* Cav. Int. J Res. Ayurveda Pharm 2013;4(3):417-419.
37. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food chemistry 1999;64(4):555-559.