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Original Article

An Investigation on the Extraction of Phytochemicals and Antimicrobial Properties of Domestic Plants Found in Southern India

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Abstract

Background and Aim: The uses of natural sources for treating various ailments have received global attraction in recent years. Flavonoids, phenolics, and alkaloid compounds present in plants satiate free radicals and scavenge oxygen. Extraction of these substances is carried out using solvents, and it varies with respect to the plant species individually. The present work was carried out to determine the best solvent for extraction. Qualitative and quantitative analyses were performed to identify the property and amount of the phytochemicals present in each plant.

Materials and Methods: Plant samples were collected and extracted using maceration and the Soxhlet extraction method. Qualitative and quantitative analyses were performed using conventional methods. Antimicrobial and antibacterial studies were performed using zone of inhibition studies based on ~~the~~ agar gel diffusion method.

Results: Of the plants samples taken for the study, the phenolic content was found to be the best one using Soxhlet methanolic leaf extracts of *Acalypha indica* (4.80mg/ml), methanolic crude extract of *Lawsome inermis* (4.7mg/ml), soxhlet water extract of *Acalypha indica* (4.35mg/ml), Soxhlet methanolic extract of *Lawsome inermis* (4.3mg/ml) and crude ethanolic extract of *Lawsome inermis* (4.0mg/ml). Crude and soxhlet ethanolic extracts of *Azadirachta indica* revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins, and steroids. Determination of vitamin C revealed that the crude methanolic extract of *Murraya koenigii* contains a higher level of vitamin C(3.7 mg/ml) content compared to the other plants and solvents used. Among the microorganism's tested, gram-negative bacteria, *Proteus mirabilis* and *Enterobacter fecalis* were found more sensitive to the plant extracts.

Conclusion: The crude methanolic extract of *Murraya koneigii* has revealed the presence of all phytochemicals except tannins. The methanolic crude extract of *Murraya koenigii* was found to have a high level of Vitamin C. Plant extracts increase the antimicrobial property which was revealed in qualitative and quantitative analysis.

Keywords: Phytochemicals, Antimicrobial, Flavonoid, Anthocyanins, Vitamin C

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Introduction

Since ancient times, human beings have been exploring the natural world, particularly plants, in order to find new antimicrobial, anti-inflammatory, anticancer, antiulcerogenic, hypoglycemic, and antioxidant herbs for curing ailments. Approximately, 80% of the world's population use traditional drugs for primary health care, which are predominantly prepared using plant extracts. In India, plant-based prescriptions comprised roughly 95% of the traditional system of Unani, Ayurveda, Homeopathy and Siddha (1).

Screening of phytochemicals is the major criterion for identifying therapeutically significant compounds such as alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids, etc. (2).This ranges from simple molecules such as phenolic acid to complex compounds like tannins (3). Flavonoids have several proven medicinal properties such as anti-inflammatory, antioxidant, anticancer and antiviral properties (4). Even though this issue has been substantially dealt with so far, the need to prepare pure extracts from the plants has not been met yet. The extracts obtained from the plants are not pure compounds, but antimicrobial results have been obtained (5)**.** Increasing failure of antimicrobial activities for the treatment of many diseases has resulted in the screening of plants to detect their potential antimicrobial activities (6).

The plants used in this study were selected based on the availability and effects of plants against microbial infections. Table 1 indicates the native Indian names the plants used. *Azadirachta indica*, which is native to India, is easily available and belongs to the family Meliaceae (7). Every part of the neem tree is medicinally valuable and could be commercially exploited. To name a few cases, dried neem leaves are effective in curing ringworm, eczema, and scabies (8). The barks and roots are used to battle against skin infections such as acne, psoriasis, scabies, eczema. Furthermore, they are used in the treatment of diabetes, cancer, heart disease, AIDS, herpes, allergies, ulcers, hepatitis, and several other diseases (9-11). Oil, seed, leaves and neem cake fight against insects, fungi and bacteria (12,13). Bark and leaves of *Azhadirachta iindica* have antifungal, antiviral, antiperiodic, anti-inflammatory, antifertility, mosquito larvicidal, spermicidal and hypoglycemic activities efficient in curing the inflammation of gingivitis and gums, sores, boils, periodontics, enlargement of spleen, malarial fever, fever after child birth, smallpox, meseals, head scald and cutaneous affections. The oil could be utilized as a contraceptive for intravaginal uses, and also for the treatment of vaginal infections (14).

Acalypha indica Linn (family Euphorbiaceae) is a weed commonly found in many regions of Asia, particularly in India. The whole plant is used as an anthelmintic and diuretic herb. Moreover, it is used for respiratory problems like asthma, bronchitis, and pneumonia (15). Powdered leaves have significant effects in curing bedsores and infected wounds (16). Tincture of fresh plant could be utilized in homeopathy in the early stage of phthisis with bloody expectorations, emaciation, and arterial hemorrhage. Khare utilized the leaves for scabies and the whole plant in the treatment of asthma, pneumonia, and bronchitis in 2007**.** This plant is used traditionally as diuretic, anthelmintic and for respiratory problems such as bronchitis, asthma, skin diseases and pneumonia (17-19).

Acalypha indica could be used in the treatment of the illnesses related to the teeth and gums, burns, toxins of plant and mixed origin, stomachache, bleeding piles, irritations, stabbing pain, wheezing and sinusitis (20)**.**

Murraya koenigii Linn.Spreng is an aromatic plant that has been widely used in India as ayurvedic medicine. Its leaves are used in the treatment of dysentery, diarrhea, and stomachache (21, 22). Traditionally, *Murraya koenigii* leaves are used in the treatment of piles, headache, stomachache, influenza, rheumatism, traumatic injury, and insect as well as snake bites. Furthermore, they are anti-vomiting, and can cure dysentery and diarrhea (23). It has already been indicated that carbazole alkaloids found in the plant cause several biological activities, including antimalarial, antioxidant, cytotoxic, anti-HIV, anti-microbial, anti-diarrheal, and anti-inflammatory activities (24, 25).

Lawsonia inermis is the scientific name of henna which belongs to the family Lythraceae. This plant is

used in traditional medicines for treating diseases such as bronchitis, menstrual disorders, hemorrhoids, rheumatism, jaundice, dysentery, leprosy and skin problems (26). Henna has also shown the antidiarrheal, diuretic, emmenagogue and abortifacient properties. Moreover, it was found to be practically nontoxic (27).

Lawsonia inermis exhibited activity against ringworm caused by fungal species such as *Microsporum gypseum* and *Trichophyton mentagrophytes* (28). *Lawsonia inermis* extract inhibited *Sindbis* virus at a minimum concentration (29). Its bark could be used in the treatment of jaundice, skin diseases and enlargement of spleen. It is chewed and kept between the teeth to treat toothache. Leaves of the plants are used against menorrhagia, headache, lumbago, bronchitis, gonorrhea, ulcers. Plants can act as virucidal agents and are used in the treatment of ringworm infections and skin related diseases.

As these plants have the ability to cure many diseases related to microorganisms, the active compound which can inhibit the growth of the pathogens has to be identified, so that a potential antimicrobial agent can be isolated.

The present work was carried out to evaluate the potential ability of the leave extracts against various microbes. Qualitative and quantitative screenings of the extracts were conducted to identify the property and amount of the phytochemicals present in each plant.

Materials and Methods

Plant Samples and Preparation of the Extracts

Acalypha indica and *Azadirachta indica* were collected from Anna University campus, Chennai India. *Murraya koenigii* and *Lawsonia inermis* were purchased from the local market, Chennai, India. All the collected samples were washed with tap water thrice and then were dried under shade. The dried samples were crushed using remi mixer grinder and kept in an airtight container until further use.

Maceration Extraction Method

Extraction was carried out by soaking 5 g of the dry powdered sample in 20 ml of ethanol, methanol and water at room temperature for 48 h, and was filtered through a Whatman filter paper No: 1. The filtrates obtained were refrigerated at 4°C until further use (30).

Soxhlet Extraction

The dried powder samples were packed in a porous cellulose thimble. The thimble was placed in an extraction chamber which was fixed to the condenser above and round the flask which contained the solvent (Ethanol, Methanol or Water). The flask was heated using a mantle and the solvent evaporated and moved up into the condenser where it was condensed as liquid that trickled into the extraction chamber containing the sample. The extraction chamber was prepared in such a way that as the solvent surrounding the sample exceeded a certain level, it overflew and dripped back down into the round bottom flask (31). This process was followed for 6 h and was repeated as the extraction process continued. The extracts were obtained and stored at 4°C (Scheme 1).

Phytochemical Screening: Qualitative Analysis

Preliminary qualitative phytochemical screening was carried out for various phytochemicals such as alkaloids, flavonoids, tannins, phenols, saponins, steroids, anthocyanins and quinone that are present in the plants. The analysis was carried out using the standard procedures adopted, alkaloid (30), flavonoids (32), steroids (33), tannins (34), phenol (35), saponins (36), anthocyanins (37), quinones (26).

Quantitative analysis

Determination of Total Phenolic Compound

The amount of the total phenolic content of the extracts was measured using the Folin-Ciocalteu reagent. Gallic acid was used as a standard, and the total phenolic content was indicated as mg/ml gallic acid equivalents (GAE). Folin-Ciocalteu reagent was sensitive to the reduction of the level of compounds, including polyphenols, leading to the production of a blue color upon the reaction which is measured spectrophotometrically (38).

Estimation of Vitamin C Content

The total vitamin content of plant extracts was measured using Folin phenol reagent. 0.5ml of the extracts was mixed with oxalic acid from the mixture 0.2-0.5ml of the sample was taken diluted to 2ml with distilled water. 0.2ml of diluted Folin reagent was added to the extract. The tubes were strenuously shaken. Ten minutes later, the measurement of the absorbance was carried out at 760nm using a UV-

visible spectrophotometer (HITACH U-2000) (39).

Test Organisms

Bacterial strains of Gram-positive organisms such as *Staphylococcus aureus* MTCC3160, *Bacillus subtilis* MTCC 441, and Gram-negative organisms like *Klebsiella pneumonia* MTCC *7162, Proteus mirabilis* MTCC 1771, *Pseudomonas aeruginosa* MTCC 424, *Enterobacter faecalis* MTCC 439 *and Escherichia coli* MTCC 443 were collected from Microbial Type Culture Collection Centre, Punjab.

Anti-Bacterial Assay

Agar Well Diffusion Method

Nutrient agar was prepared by weighing 0.5g of peptone, 0.3g of beef extract/yeast extract, 0.5g of sodium chloride and 1.5g of agar in 100ml of distilled water. The prepared media was autoclaved at 121°C for 15 minutes. The media was poured into the plates and allowed to solidify. Inoculum of 24 hours culture was swabbed (rubbed) on the plate with the help of cotton swab. Wells were punched on each plate using sterile borer. Each plant extract was added to wells which were punched. The plates were incubated in an upright position at 37°C for 24 hours in an incubator. The antimicrobial activity was determined measuring the diameter of the zone of inhibition using Hi media zone measuring scale (40).

Minimum Inhibition Concentration (MIC) Assay

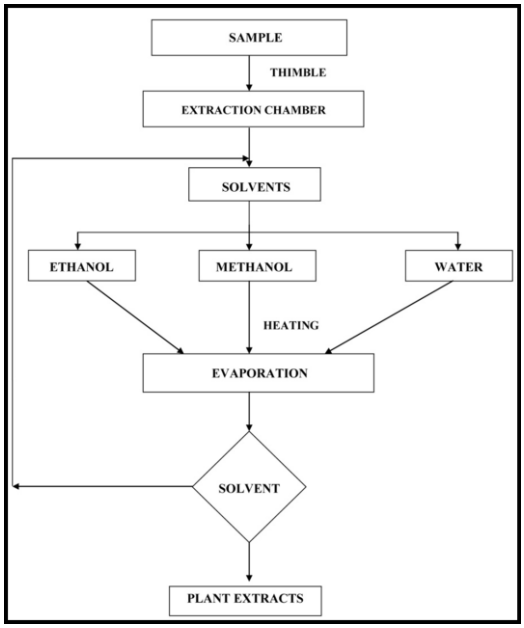
Minimum Inhibition Concentration (MIC) for plant extract was measured by serial dilution. Stock solutions were prepared by adding dimethyl sulphoxide to plant extracts to make a concentration of 250mg/ml added to the first column in 96 well microplates. The suspension made of bacterial colony was approximated to 5×10^5 cfu/ml. From this suspension, 100 μl was inoculated into each well. One well for growth and one well for sterility were maintained for each strain. The microplates were incubated for 24hours at 37°C. MIC was determined by adding 30 μl of 0.02% p-iodonitrotetrazolium chloride (p-INT). It was again incubated at 37°C for 30 minutes. Bacteria metabolizes p-INTand changed the color to pink. No color change indicated no growth. This was read as MIC values,

Determination of Minimum Bactericidal Concentration (MBC)

Microtitre broth dilution method was adopted to find minimum bactericidal concentration (MBC) .MBC was performed on all the extracts. Ten microliters were taken from the well obtained from the MIC experiment (MIC value) and two wells above the MIC value well and were spread on plates. The number of colonies was counted after 18–24 h of incubation at 37°C. The lowest concentration of the extract that showed no bacterial growth after incubation was observed. The concentration of the sample that generates less than 10 colonies was measured as MBC value. Every experiment was replicated at least three times

Results and Discussion

The phytochemical screening indicated the existence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, anthocyanins and quinone in the plant extracts taken for our study. Every individual extract based on the solvent used showed variation in properties. The aqueous crude extract of *Acalypha indica* had shown the presence of phytochemicals such as alkaloids, flavonoids, tannins, phenols, saponin and quinones. The aqueous soxhlet extract showed the presence of steroids and absence of tannin. The methanolic extract of both crude and soxhlet revealed the presence of phenol, quinones and saponins. Crude and Soxhlet ethanolic extracts exhibited the presence of flavonoids, phenols and saponins.



Scheme 1. Soxhlet Extraction.

Table 1: Regional names of selected plants*.

S. No	Botanical names	Regional names of plants*					
		Sanskrit	Hindi	Tamil	Telugu	Malayalam	English
1	*Azadirachta indica*	Arista, Picumarda	Nim, Nimb	Veppai, Vembu	Vemu, Vepa	Vempu, Aruveppu	Margosa tree
2	*Acalypha indica*	Haritamajari	Kuppi, Aammaabhaaji	Kuppaimeni	Kuppichettu, Kuppinta, Muripindi	Kuppameni	Indian acalypha
3	*Murraya konegii*	Saurabhanimba	Mitha neem, Kadhi Patta, Kadi Patta	Karivempu, Karuveppilei	Karivepaku, Karivemu	Kariveppu	Curry leaf
4	*Lawsonia inermis*	Nil Madayantika	Mehandi	Marudum	Gorinta, Korate, Madarangi	Mailanelu	Henna

*Data from Ayurvedic pharmacopoeia of India Vol: 2, 4 and 6

Table 2: Total Phenolic Content of Plant Extracts.

Plant Extract	*Acalypha indica*	*Azadirachta indica*	*Lawsonia inermis*	*Murraya koenigii*
	mg/ml	mg/ml	mg/ml	mg/ml
Ethanol Crude	0.4	1.2	4	1.4
Methanol Crude	2.5	2.3	4.7	1.9
Water Crude	1.9	0.6	3.5	0.3
Ethanol Soxhlet	1.7	2.5	3.25	1.3
Methanol Soxhlet	4.8	3.1	4.3	2.4
Water Soxhlet	4.35	2.3	2.25	0.7

Several studies have been conducted to test the presence of phytochemicals with crude and solvent based extracts. However, there seems to be variations in aqueous crude and Soxhlet extracts of *Azadirachta indica* that exhibited the presence of alkaloids, flavonoids, and phenols. The crude methanolic extract contained phytochemicals such as alkaloids, flavonoid, tannins, phenols, and steroids while in soxhlet methanolic extract alkaloids, flavonoid and phenols are present. Crude and Soxhlet ethanolic extracts of *Azadirachta indica* indicated the existence of alkaloids, flavonoids, tannins, phenols, saponins, and steroids.

In the case of *Lawsonia inermis* aqueous crude extract showed the existence of tannins, phenols, steroids, anthocyanins, and absence of other compounds. The aqueous soxhlet showed the presence of alkaloids, flavonoids tannins, phenols, steroids, and anthocyanins. The methanolic crude extract showed the presence of flavonoids, tannins, phenols, steroids, and anthocyanins, whereas in the methanolic Soxhlet extract showed the presence of flavonoids, tannin, phenol and anthocyanins and absence of steroids which were present in the crude extract. The crude ethanolic extract of *Lawsonia inermis* showed the presence of phytochemicals such as alkaloids,

Table 3: Vitamin C content of Plant Extracts.

Plant Extract	*Acalypha indica*	*Azadirachta indica*	*Lawsonia inermis*	*Murraya koenigii*
	mg/ml	mg/ml	mg/ml	mg/ml
Ethanol Crude	0.2	0.6	0.84	1.8
Methanol Crude	1.23	2.2	1.3	3.7
Water Crude	0.63	0.36	0.73	0.94
Ethanol Soxhlet	0.56	0.48	0.54	0.84
Methanol Soxhlet	0.89	0.67	0.68	2.4
Water Soxhlet	0.32	0.24	0.42	0.63

Table 4: Minimum Inhibition Concentration (MIC) Assay.

Species.	*Acalypha indica*		*Azadirachta indica*		*Lawsonia inermis*		*Murraya koenigii*	
	Methanol Extract	**Ethanol Extract**	**Methanol Extract**	**Ethanol Extract**	**Methanol Extract**	**Ethanol Extract**	**Methanol Extract**	**Ethanol Extract**
Pseudomonas sps	2.39	15.67	3.46	18.12	4.51	17.94	3.87	18.55
Proteus sps,	92.36	24.18	91.27	22.57	94.06	21.85	90.37	22.03
Klebsiella sp.	91.67	2.06	94.49	2.81	90.55	2.44	93.45	2.86
Bacillus sps	5.65	51.23	5.49	54.29	5.67	53.14	5.74	54.97
Escherichia coli	89.34	90.23	88.57	94.25	88.27	91.19	84.35	90.32
Enterobacter sp	22.77	74.26	23.64	75.21	23.41	76.99	23.57	78.64
Staphylococcus sps	90.31	36.87	91.22	39.12	94.34	37.64	92.33	34.52

flavonoids, tannins, phenols, steroids, and anthocyanins, while the Soxhlet revealed the existence of flavonoid, tannin and phenols and absence of other phytochemicals.

Murraya koenigii aqueous crude extract showed the absence of all the phytochemicals tested and in the case of Soxhlet extract showed the presence of flavonoids and phenols. The methanolic crude extract showed the presence of all the compounds except tannin, in the Soxhlet extract along with tannin, steroids, anthocyanins and quinones were also absent. The crude ethanolic extract showed the presence of alkaloids, flavonoids, phenols, saponins and steroids while Soxhlet showed the presence of flavonoids, phenols, saponins and steroids. Among the above results, methanol and ethanol extracts of plants indicated the existence of most of the phytochemicals while in the aqueous extract some of them were absent.

Estimation of the Total Phenolic Content

The results of the total phenolic content that were obtained have been presented in Table 2. Gallic acid was used as the standard for the determination of the total phenolic content (37). In *Acalypha indica*, the methanolic Soxhlet extract showed the higher total phenolic content of 4.8mg/ml, Soxhlet aqueous extract with 4.35mg/ml, methanolic crude extract (2.5mg/ml), water crude extract (1.9 mg/ml), ethanolic soxhlet extract (1.7mg/ml) and ethanolic crude extract (0.4 mg/ml).

Methanolic extract of *Azadirachta indica* showed the higher amount of phenolic content of 3.1mg/ml, the

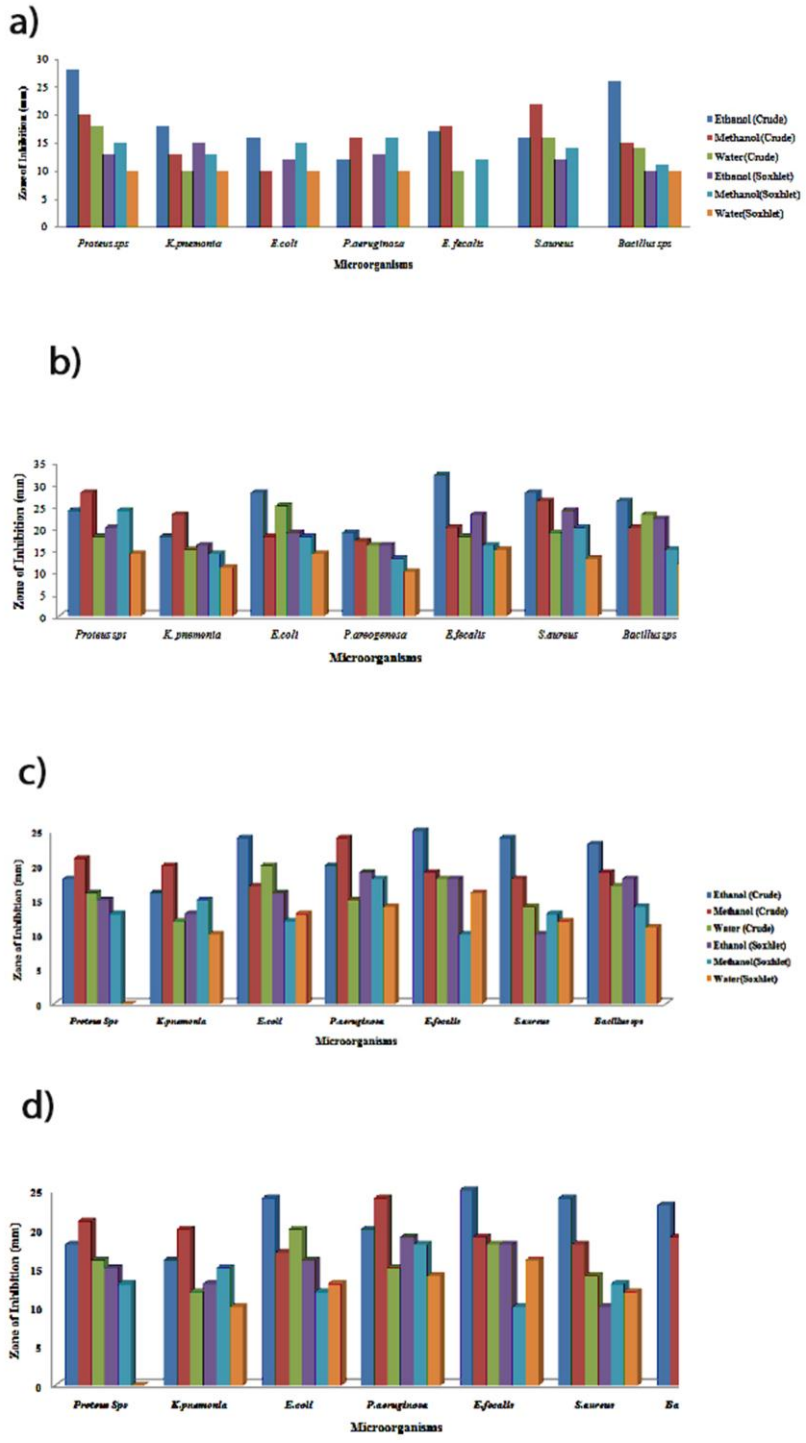


Figure 1. Zone of Inhibition for different crude extracts on microorganisms a) *Acalypha* Indica b) *Azadirachta* Indica c) *Lawsonia inermis d) Murraya koenigii*.

second higher content was seen in ethanolic soxhlet extract with 2.5 mg/ml followed by methanolic crude extract (2.3 mg/ml), aqueous soxhlet extract (2.3 mg/ml) ethanolic crude extract (1.2 mg/ml) and

aqueous crude extract (0.6 mg/ml).

Lawsonia inermis methanolic crude extract showed higher amount of phenolic content of 4.7mg/ml, the soxhlet methanolic extract showed second higher phenolic content (4.3 mg/ml) and then ethanolic crude extract showed phenolic content of 4mg/ml. The aqueous crude, ethanolic soxhlet and aqueous soxhlet extract showed 3.5 mg/ml, 3.25 mg/ml and 2.25 mg/ml concentration of phenolic content respectively.

In *Murraya koenigii*, a higher amount of the phenol content was seen in the methanolic Soxhlet extract (2.4 mg/ml), methanolic crude extract (1.9 mg/ml), ethanolic crude extract (1.4mg/ml), ethanolic Soxhlet extract (1.3 mg/ml), aqueous Soxhlet extract (0.7 mg/ml) and aqueous crude extract (0.3 mg/ml).

Based on the release of the phenolic compound in the extract, the amount of the total phenolic content varies. On testing each plant extract, the highest concentrations of the phenolic content were found in the Soxhlet methanolic leaf extracts of *Acalypha indica* (4.80 mg/ml), methanolic crude extract of *Lawsome inermis* (4.7 mg/ml), Soxhlet water extract of *Acalypha indica* (4.35 mg/ml), Soxhlet methanolic extract of *Lawsome inermis* (4.3 mg/ml) and crude ethanolic extract of *Lawsome inermis* (4.0 mg/ml).

Estimation of Vitamin C (Ascorbic Acid)

We used ascorbic acid as the standard to determine the level of vitamin C in various plant extracts. The results obtained for vitamin C have been presented in Table 3. Crude methanolic extract of *Acalypha indica* showed higher vitamin C content (1.23 mg/ml), and the lowest content was obtained from the crude ethanolic extract (0.2 mg/ml). The extracts from the ethanolic Soxhlet, methanolic soxhlet, aqueous soxhlet and aqueous crude had 0.56 mg/ml, 0.89mg/ml, 0.32 mg/ml and 0.63 mg/ml of vitamin C, respectively.

Azadirachta indica crude methanolic extract exhibited the highest vitamin C content of 2.2 mg/ml, and the second highest content of vitamin C was exhibited by the Soxhlet methanolic extract (0.67 mg/ml). Finally, the lowest content was observed in the aqueous Soxhlet extract (0.24mg/ml).

In *Lawsonia inermis*, the crude methanolic extract exhibited a higher vitamin C content of 1.3mg/ml compared to the crude ethanolic extract (0.84 mg/ml), crude aqueous extract (0.73 mg/ml), Soxhlet ethanolic extract (0.54 mg/ml), methanolic extract (0.68 mg/ml) and aqueous extract (0.42 mg/ml).

Methanolic crude and Soxhlet extracts of *Murraya koenigii* showed higher concentrations of vitamin C, i.e., 3.7mg/ml and 2.4mg/ml respectively. The ethanolic crude showed a higher content of 1.8 mg/ml of vitamin C, crude aqueous showed 0.94 mg/ml, and Soxhlet ethanolic and aqueous showed 0.84 mg/ml and 0.63 mg/ml respectively.

Antimicrobial Assay

From Figure 1a, it can be ascertained that the crude ethanolic extract of *Acalypha indica* exhibited the maximum antimicrobial activity against the pathogens used. It showed a greater level of activity against *Proteus sps, Bacillus sps* and *Staphylococcus aureus*. Among the Soxhlet extracts, the methanolic extract exhibited a higher level of antimicrobial activity against *Pseudomonas aeruginosa, Escherichia coli* and *Proteus.*

Crude methanolic extract of *Azadirachta indica* showed the maximum antimicrobial activity against *Proteus sps, Staphylococcus sps, Klebsiella sps, Enterobacter sps* and *Bacillus sps.* Of all the Soxhlet extracts, the methanolic extract indicated the maximum zone of inhibition against all the pathogens. The ethanolic extract showed a higher potency of inhibition on staphylococcus aureus compared to the methanolic extract (Figure 1 b).

In *Lawsonia inermis*, the crude methanolic extract showed the maximum activity against *Proteus sps, Klebsiella pneumonia* and *Staphylococcus aureus*. The ethanolic extract also showed potential antimicrobial activity against all the pathogens used compared to the aqueous extracts. The crude methanolic Soxhlet extract illustrated the maximum antimicrobial activity compared to the ethanolic and aqueous extracts (Fig 1c).

The extracts of Murraya koenigii showed that the crude ethanolic extract could exhibit the maximum effect against pathogens such as *Enterobacter sp, Staphylococcus sps, Escherichia coli, Bacillus sps* and *Pseudomonas sps.* The crude methanolic extract exhibited effective activity against all the pathogens. In the Soxhlet extracts, the ethanolic extract exposed a higher potency of activity compared to the other two solvents (Figure 1d).

The MIC results of the plant extract confirmed its zones of inhibition of antibacterial activity in the case of *K. pneumonia*. However, with regard to other bacteria, there was a slight agreement with the primary zone of inhibition mean diameter which is indicative of having higher inhibition and lower concentration. The MIC and MBC of the tested bacteria were almost identical for *proteus sp.*, *Klabsiella sp.*and *E. coli* as indicated in Table 4.

The presence of phytochemicals and flavonoids improves the antioxidant property. Flavonoids are hydroxylated phenolic substances which are synthesized by plants in reaction to microbial infection, and it is not astounding that they have been found *in vitro* to be influential antimicrobial substances capable of acting against a remarkable variety of microorganisms. Their activity is possibly because of their ability to form complex with extracellular and soluble proteins. Moreover, they form complex with bacterial cell walls. The extracts in our results showed the presence of alkaloids, flavonoids, and phenols.Studies on different parts of the plants of *Acalypha indica*, and the results are highly in agreement with the obtained findings. The difference in the results with previous research on alkaloids, flavonoids and phenols might be due to the polarity of the solvents used (26). On the polarity of the solvents, methanol and ethanol extracts showed greater numbers of phytochemical components than water extracts. Although aqueous extracts contain some of the phytochemicals tested, their absence might be due to the low concentration and release of phytochemicals in the solvents. Similar results were reported by Oseni Lateef Adebayo *et.al* in 2012.

In the case of the total phenolic content, based on the release of the phenolic compound in the extract, the amount of the total phenolic content varies. With the results obtained, it can be stated that the highest concentrations of the phenolic content were found in the Soxhlet methanolic leaf extracts of *Acalypha indica* (4.80mg/ml), methanolic crude extract of *Lawsome inermis* (4.7mg/ml), Soxhlet water extract of *Acalypha indica* (4.35mg/ml), Soxhlet methanolic extract of *Lawsome inermis* (4.3mg/ml) and crude ethanolic extract of *Lawsome inermis* (4.0mg/ml). The results indicated that the methanolic extract could have a better extraction advantage over crude extracts. From the phytochemical analysis, the methanolic extracts showed the presence of tannins and a higher total phenolic content.

In recent studies, there has been an increasing interest in the investigation of vitamins present in whole plants for their own growth as well as for utilizing them for humans naturally (32). Owing to robust biological activities, they may possibly act as scavengers. The results of the determination of vitamin C revealed that the crude methanolic extract of *Murraya koenigii* contained a higher level of vitamin C (3.7 mg/ml) content compared to other plants and solvents used. The least content of vitamin C was observed for the crude ethanolic extract of *Acalypha indica* (0.2 mg/ml). Vitamin C is a water-soluble antioxidant. There might be a decrease in its levels. However, the level of vitamin C was found to be effective in the treatment of diseases.

The crude methanolic extract showed a higher potency of antimicrobial activity against the microorganism than other extracts. Choudhury *et al*. evaluated the inhibitory effects with different solvents, and their results are mostly comparable with our results (21). Though Soxhlet extracts showed the presence of the most phytochemicals, these extracts exhibited a lower level of antimicrobial activity, which might be due to the hindrance of other compounds in the extracts (40). Among the microorganism's Gram-negative bacteria, *Proteus mirabilis* and *Enterobacter fecalis* were found more sensitive to the plant extracts. A similar type of observation was reported where the Gram-negative organism was found to have a greater resistance (20).

The polyphenols present in plant materials delay the microorganism growth by causing interruptions in the cell wall of the microorganism, thereby retarding its growth. Our results also confirm the antimicrobial activity. Dewi kusrini *et al*. also conducted a research with regard to phenol determinations and reported the results of the antitoxicity assay using the DPPH method (39). They found that it depends on the species tested, but not uniformly for all species. Moreover, the antimicrobial activity was different against the species tested. The results confirm evidence of the potential sources of natural antioxidant and antimicrobial agents. The lower activity of aqueous extracts might be due to the low concentration of antimicrobial

compounds. Active compounds against microorganisms such as aromatic or saturated organic compounds are more often acquired via the ethanol or methanol extraction (17). Based on the detected bioactive compounds in individual plants, each plant can be effectively utilized for different uses found on the extraction process.

The plant extract of methanol had the maximum MIC of 94.06 mg/ml for *proteus sp.*), and the minimum MIC value was 2.39 mg/ml for *pseudomonas*. This actually shows the difference which was observed in the zone of inhibition. Nonetheless, the results predominantly agreed well with the zone of inhibition results. The MBC value also had the same order of 3.05 mg/ml, 92.38mg/ml, 5.75 mg/ml, 89.06 mg/ml, 24.16mg/ml and 91.04 mg/ml, respectively for the methanol extracts for the tested organisms (*Pseudomonas* sp. *Proteus sps, Klebsiella sp., Bacillus sp. E.Coli, Enterobacter* and *Staphylococcus sp.).* The MBC value for ethanol extracts were obtained as 15.55 mg/ml, 23.02mg/ml, 5.15 mg/ml, 51.06 mg/ml, 91.43mg/ml, 73.16mg/ml and 35.64 mg/ml respectively for the tested organisms (*Pseudomonas* sp. *Proteus sps, Klebsiella sp., Bacillus sp .E.Coli, Enterobacter* and *Staphylococcus sp.).* Almost the same results were obtained for all the plants taken for study in the case of MBC. According to Kang et al., minimum bactericidal effects were exhibited with diverse degrees in methanol extracts (23). They stated that the methanol extracts showed significant antimicrobial activity, whereas in our study the ethanol extract exhibited a higher level of antimicrobial activity. This might be due to the variety of plant extracts taken for the study.

Conclusion

The methanolic and ethanolic extracts of the plants were found to have greater numbers of phytochemicals compared to the aqueous extract. The crude methanolic extract of *Murraya koneigii* revealed the presence of all phytochemicals except tannins. Furthermore, the methanolic crude extract of *Murraya koenigii* was found to have a high content of Vitamin C (ascorbic acid). Various active compounds present in the plant extracts increased the antimicrobial property of the plant extracts which were revealed in qualitative and quantitative analyses. Both the MIC and MBC results well agreed with the zone of inhibition studies to confirm the results for the antimicrobial activity. Further investigation of active compounds is essential to isolate and describe the distinctive features of the bioactive compounds to produce new antimicrobial drugs.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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