

# Phytochemical Analysis and Antimicrobial Activity of South Indian - An Original Plants

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**Abstract:** The phytochemical profile and antimicrobial activity of leaf extracts obtained from *Acalypha indica*, *Azadirachta indica*, *Lawsonia inermis* and *Murraya koenigii* were studied. In the present work, plant extracts were prepared using ethanol, methanol and water as solvents. Crude and soxhlet method was used for extraction process. Qualitative phytochemical analysis was done to reveal the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, and anthocyanins. Quantitative analysis were done for the estimation of phenolic content and vitamin C. Soxhlet methanolic leaf extracts of *Acalypha indica* (4.80mg/ml) showed a high level of phenolic content and Vitamin C content was found to be high in methanolic crude and soxhlet extract of *Murraya koenigii* (3.7mg/ml and 2.4mg/ml). The antimicrobial assay was carried out by agar disk diffusion method against seven bacterial strains. Gram-positive organisms such as *Staphylococcus aureus*, *Bacillus subtilis* and gram-negative organisms like *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Enterobacter faecalis* and *Escherichia coli* were used. The antimicrobial activities of each extract were assessed by measuring the diameter zone of inhibition. The plant extracts tested were highly effective against *Proteus mirabilis* and *Enterobacter faecalis*. Compared to aqueous extracts, methanolic and ethanolic extracts showed more antimicrobial activity. Methanolic extract of *Lawsonia inermis* showed higher zone of inhibition compare to other extracts

**Keywords:** Phytochemicals, Natural products, Flavonoid, Anthocyanins, Vitamin C, Antioxidants.

## 1. Introduction

Since ancient times, people have been exploring the nature, particularly plants, in search of new antimicrobial, anti-inflammatory, anticancer, antiulcerogenic, hypoglycemic, antioxidant drugs for curing ailments. This has resulted in the use of a large number of medicinal plants with curative properties to treat various diseases such as microbial infections, cancer, ulcer, hypoglycemia, hyperglycemia, etc. Nearly 80% of the world's population relies on traditional medicines for primary health care, most of which involves the use of plants extracts. In India, almost 95% of the prescriptions were plant-based in the traditional system of Unani, Ayurveda, Homeopathy and Siddha [32].

The main source which shows the curative properties of plants are the phytochemicals which are produced through normal metabolism. These phytochemical constituents are the

basic source, for the establishment of several pharmaceutical products. Hence screening of phytochemicals is the major criteria for identifying of therapeutically important compounds like alkaloids, flavonoids, phenolic compounds, saponins, steroids, tannins, terpenoids etc. [38]. The present work focuses on search for phenolic compound as it is one of the most important groups of all other components present in the plant extracts. It ranges from simple molecules such as phenolic acid to complex compound such as tannins [14]. Flavonoids have several proven medicinal properties such as anti-inflammatory, antioxidant, anticancer and antiviral properties [19].

Even though large amount of work has been carried out, a need for pure extract from the plants is still unfold. The extracts are not pure compounds and in spite of it, antimicrobial results are obtained [20]. Increasing failure of chemotherapeutics and antibiotic resistance exhibited by microbial pathogens has led to the screening of plants for their potential antimicrobial activity [3].

In many of the studies only crude type of extraction was done where the plant material was soaked with the solvent and filtered or through soxhlet extraction. In the present study, both the methods of extraction were done to study the potential of the plant extracts.

The plants were selected based on the availability and property of plants against microbial infections. *Azadirachta indica* which are native to India and easily available belongs to the family Meliaceae is of great medicinal value [43]. Every part of the neem tree has some medicinal value and commercially used. The dried neem leaf is effective to cure ringworm, eczema and scabies [37]. The barks and roots of neem are used to fight against skin infections such as acne, psoriasis, scabies, eczema and treats diabetes, cancer, heart disease, AIDS, herpes, allergies, ulcers, hepatitis and several other diseases. [5, 18, 35]. A powerful insecticide, insect and nematode repellent; oil, seed, leaves and cake against insects, fungi, bacteria, viruses. Fruit aromatic oil used for malaria, skin diseases, stomach ulcers, worms, rheumatism, leprosy and eczema; *neem* oil has contraceptive properties, application of the oil from the seeds to both male and female genital organs [40]. Mitochondrial injury in mice and poisonous in high dosages in the consuming of neem oil [33].

Bark and leaves of *Azadirachta indica* has antifungal,

antiviral, antiperiodic, anti-inflammatory, antifertility, mosquito larvicidal, spermicidal, hypoglycemic, used in the inflammation of gingivitis, gums, sores, boils, peritonitis, enlargement of spleen, malarial fever, fever after child birth, smallpox, measles, head scald and cutaneous affections. Oil was used as contraceptive for intravaginal uses, and for the treatment of vaginal infections [21].

*Acalypha indica* Linn (family Euphorbiaceae) is a common weed found in many parts of Asia especially in India. Whole plant is used as anthelmintic, diuretics and for respiratory problems like asthma, bronchitis and pneumonia [39]. The juice extracted from leaves is used to cure ringworms, rheumatoid arthritis and scabies. Powdered leaves have potential in curing bedsores and infected wounds [28]. Tincture of fresh plant is used in homoeopathy for incipient phthisis with bloody expectorations, emaciation and arterial haemorrhage. Leaves used in scabies, whole plant are used in treatment of asthma, pneumonia and bronchitis [21].

This plant is used traditionally as diuretic, anthelmintic and for respiratory problems such as bronchitis, asthma and pneumonia [4]. The dried leaves of *Acalypha indica* was made into a soft paste to treat bedsores and wounds and the juice of *Acalypha indica* is added to oil or lime and used to treat skin disorders [7].

*Acalypha indica* cures diseases of the teeth and gums, burns, toxins of plant and mixed origin, stomach pain, bleeding piles, irritations, stabbing pain, wheezing and sinusitis [15].

*Murraya koenigii* Linn. Spreng is an aromatic plant that has been widely used in India as ayurvedic medicine. Its leaves are used to treat dysentery, diarrhoea, and stomach ache [37]. Leaves, root and bark are good tonic and carminative. Renal pain can be relieved by intake of root juice [42]. Traditionally *Murraya koenigii* leaves are used for the treatment of piles, headache, stomach ache, influenza, rheumatism, traumatic injury, insect, snake bites, anti-vomiting, curing dysentery and diarrhea [22].

It has been reported that carbazole alkaloids present in the plant is responsible various biological activities including antimalarial, anti-oxidant, cytotoxic, anti-HIV, anti-microbial, anti-diarrheal, and anti-inflammatory activities [29, 41].

*Lawsonia inermis* is a 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, and leprosy and skin problems [17]. Henna has been found to exhibit antibacterial, antifungal and dermatological properties. It is useful in colouring of skin, scalp and nails etc. Henna has also shown antidiarrhoeal, diuretic, emmenagogue and abortifacient properties and is found to be practically non toxic [25].

*Lawsonia inermis* exhibited activity against ringworm caused by fungal species such as *Microsporum gypseum* and *Trichophyton mentagrophytes* [36]. *Lawsonia inermis* extract inhibited *Sindbis* virus at a minimum concentration [26]. Bark

for jaundice, skin diseases and enlargement of spleen; bark chewed and kept between the teeth to treat toothache. Leaves of the plants are used for menorrhagia, headache, lumbago, bronchitis, gonorrhoea, ulcers. The leaf juice mixed with water and sugar is consumed in spermatorrhea. Plant act as vibriocidal agents, and used in the treatment of ringworm infections and skin related diseases. It also used as veterinary medicine, crushed leaves are eaten for acidity, diarrhea and stomach problems; paste of the leaves were applied for shoulder pain and foot diseases. Sacred plant, ritual leaf paste is used to dye nails and palms of hand [40].

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

The present work is carried out to evaluate the potential of the leave extract against various microbes. Qualitative and quantitative screening of those extracts was obtained to identify the property and amount of the phytochemicals present in each plant.

## 2. Materials and method

### A. Plant Samples and Preparation of Extract

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

### B. Crude Extract

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

### C. 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 flask which contains the solvent (Ethanol, Methanol and Water). The flask was heated using a mantle and the solvent evaporates and moves up into the condenser where it is condensed as a liquid that trickles into the extraction chamber containing the sample. The extraction chamber is designed so that when the solvent surrounding the sample exceeds a certain level it overflows and drips back down into the round bottom flask. This process was continued for 6 h and repeated extractions were done. Extracts were obtained and stored at 4 °C (Scheme 1).



Fig. 1. Soxhlet Extraction

#### D. Phytochemical Screening:

- Qualitative analysis:** Preliminary qualitative phytochemical screening was carried for various phytochemicals such as alkaloids, flavonoids, tannins, phenols, saponins, steroids, anthocyanins and quinone that is present in the plants.
- Alkaloid:** 2 mL of filtrate was treated with 2-3 drops of Mayer's reagent; formation of cream-coloured precipitate indicates the presence of alkaloids [27].
- Flavonoids:** 5 ml of dilute ammonia solution were added to a portion of each plant extract followed by addition of concentrated H<sub>2</sub>SO<sub>4</sub>. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing [12].
- Steroids:** 1 ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added drop wise on the sides of the test tube. The upper layer turns red, while the sulphuric acid layer shows a yellow greenish fluorescence. This indicates the presence of steroids [23].
- Tannins:** 2 ml of extract was added to few drops of 1% lead acetate. Yellowish precipitate indicates the presence of tannins [23].
- Phenol:** The extract will be treated with 10% sodium chloride solution; appearance of cream colour indicates the presence of phenolic compounds [31].
- Saponins:** 5 ml of extract was mixed with 20 ml of distilled water and then shaken in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins [11].
- Anthocyanins:** 2 ml of aqueous extract is added to 2 ml of 2N HCl and ammonia. The change of colour from pink-red to bluish-violet indicates the presence of anthocyanins [16].
- Quinones:** Few drops of 1 N sodium hydroxide solution were mixed with 1 ml of each extracts in test tubes. Test tubes were observed for the appearance of red colour indicating the presence of quinines [17].

#### E. Quantitative analysis

##### Determination of Total Phenolic Compound

The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent. Gallic acid was used as a standard and the total phenolic was expressed as mg/ml gallic acid equivalents (GAE). The standard calibration curve was plotted. 1ml of standard solution of concentration 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5 and 5mg/ml of gallic acid was prepared in methanol. 1 ml of each sample was introduced into test tubes and mixed with 2.5ml of a 10 fold dilute Folin- Ciocalteu reagent and 2ml of 7.5% sodium carbonate. The tubes were allowed to stand for 30 minutes at room temperature before the absorbance was read at 760nm using UV-Visible spectrophotometer. As Folin-Ciocalteu reagent was sensitive to reducing compounds including polyphenols, producing a blue color upon reaction which is measured spectrophotometrically [30].

##### Estimation of vitamin C

Vitamin C (ascorbic acid) is one of the well-known antioxidants which acts as a reducing agent to reverse oxidation in liquid. Ascorbic acid has an impact on cardiovascular diseases, hypertension and diabetes. The total vitamin content of plant extracts was estimated using Folin phenol reagent. 0.5ml of 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 vigorously shaken. After 10 min absorbance was measured at 760nm using UV-Visible spectrophotometer (HITACH U-2000) [6].

#### F. Anti-Bacterial assay

##### 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 used.

##### 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. Antimicrobial activity was determined by measuring the diameter of zone of inhibition using Himedia zone measuring scale.

Table 1  
Regional names of selected plants\*

S. No.	Botanical names	Regional names of plants*			
		Sanskrit	Tamil	Hindi	English
1	<i>Azadirachta indica</i>	Arista, Picumarda	Veppai, Vembu	Nim, Nimb	Margosa tree
2	<i>Acalypha indica</i>	Haritamajari	Kuppaimeni	Kuppi, Aammaabhaaji	Indian acalypha
3	<i>Murraya koenigii</i>	Saurabhanimba	Karivempu, Karuveppilei	Mitha neem, Kadhi Patta, Kadi Patta	Curry leaf
4	<i>Lawsonia inermis</i>	Nil Madayantika	Marudum	Mehandi	Henna

Table 2  
Total Phenolic Content of Plant Extracts

Plant Extract	<i>Acalypha indica</i>	<i>Azadirachta indica</i>	<i>Lawsonia inermis</i>	<i>Murraya koenigii</i>
	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

### 3. Results and discussion

#### A. Phytochemical screening

The phytochemical screening showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, anthocyanins and quinone in the plant extracts. 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 extract exhibited the presence of flavonoids, phenols and saponins.

Both aqueous crude and soxhlet extract of *Azadirachta indica* exhibited the presence of alkaloids, flavonoids and phenols. The crude methanolic extract showed the presence of phytochemicals such as alkaloids, flavonoid, tannins, phenols and steroids while in soxhlet methanolic extract alkaloids, flavonoid and phenols are present. Crude and soxhlet ethanolic extract of *Azadirachta indica* revealed the presence of alkaloids, flavonoids, tannins, phenols, saponins, and steroids.

In the case of *Lawsonia inermis* aqueous crude extract showed the presence 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 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, flavonoids, tannins, phenols, steroids and anthocyanins, while soxhlet showed the presence 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 case of soxhlet extract showed the presence of flavonoids and phenols. Methanolic

crude extract showed the presence of all the compounds except tannin, in 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 showed the presence of most of the phytochemicals while in aqueous extract some of them were absent. This difference in the results obtained might be due to the polarity of the solvents used. Based on the polarity of the solvents used, methanol and ethanol extracts showed more number of phytochemical components than water extracts. Although aqueous extracts contain some of the phytochemicals tested for, their absence might be due to low concentration and release of phytochemicals in the solvents. A similar type of results was reported by Adebayo Oseni Lateef *et. al.* [1] in 2012.

#### B. Estimation of total phenolic content

Gallic acid was used as a standard for the determination of total phenolic content [30]. The results of total phenolic content are presented in Table 2. In *Acalypha indica*, the methanolic soxhlet extract showed higher total phenolic content of 4.8mg/ml, the 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 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 a higher amount of phenolic content of 4.7mg/ml, the soxhlet methanolic extract showed second higher phenolic content(4.3mg/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

Table 3  
Vitamin C content of Plant Extracts

Plant Extract	<i>Acalypha indica</i>	<i>Azadirachta indica</i>	<i>Lawsonia inermis</i>	<i>Murraya koenigii</i>
	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

mg/ml and 2.25 mg/ml concentration of phenolic content respectively.

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

Based on the release of phenolic compound in the extract the amount of the total phenolic content varies. On testing, each plant extract the highest concentration of phenolic content was 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).

#### C. Estimation of Vitamin C (Ascorbic Acid)

Ascorbic Acid was used as a standard for the determination of vitamin C in various plant extracts. The results of Vitamin C were tabulated (Table 3). Crude methanolic extract of *Acalypha indica* showed higher vitamin C content (1.23mg/ml) and the lowest content was obtained from the crude ethanolic extract (0.2mg/ml). The extracts from ethanolic soxhlet, methanolic soxhlet, aqueous soxhlet and aqueous crude had 0.56 mg/ml, 0.89mg/ml, 0.32mg/ml and 0.63 mg/ml of vitamin C respectively.

*Azadirachta indica* crude methanolic extract exhibited the higher vitamin C content of 2.2mg/ml, the second higher content of vitamin C was exhibited by soxhlet methanolic extract (0.67mg/ml) and the least content was observed in aqueous soxhlet extract (0.24mg/ml).

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

Methanolic crude and soxhlet extract of *Murraya koenigii* showed a higher concentration of vitamin C, 3.7mg/ml and 2.4mg/ml respectively. The ethanolic crude showed higher content of 1.8 mg/ml of vitamin C, crude aqueous showed 0.94 mg/ml, soxhlet ethanolic and aqueous showed 0.84 mg/ml and 0.63 mg/ml respectively.

The results in the determination of vitamin C revealed that the crude methanolic extract of *Murraya koenigii* contains higher vitamin C (3.7 mg/ml) content upon other plants and

solvents used. The least content of vitamin C was shown by crude ethanolic extract of *Acalypha indica* (0.2 mg/ml).

#### D. Antimicrobial assay

From the Fig. 1(a) the crude ethanolic extract of *Acalypha indica* revealed 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 higher antimicrobial activity against *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus*.

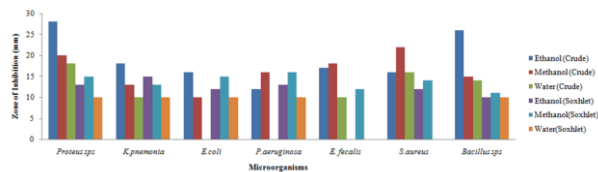
Crude methanolic extract of *Azadirachta indica* showed maximum antimicrobial activity against *Proteus sps*, *Staphylococcus sps*, *Klebsiella sps*, *Enterobacter sps* and *Bacillus sps*. Of all the soxhlet extracts, methanolic extract indicated a maximum zone of inhibition against all the pathogens.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

From the result obtained for *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 as compared to the aqueous extracts. Crude methanolic soxhlet extract illustrated maximum antimicrobial activity than ethanolic and aqueous extracts (Fig. 1 (c)).

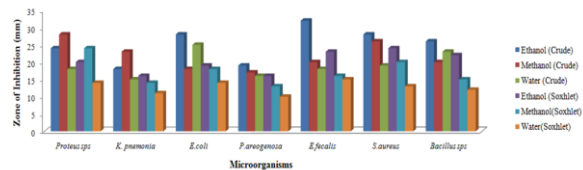
The extracts of *Murraya koenigii*, crude ethanolic extract revealed maximum effect against pathogens such as *Enterobacter sp*, *Staphylococcus sps*, *Escherichia coli*, *Bacillus sps* and *Pseudomonas sps*. The crude methanolic extract exhibited effective activity against all the pathogens. In soxhlet extracts, ethanolic extract exposed higher potency of activity than the other two solvents (Fig. 1 (d)).

In the present study, the crude methanolic extract showed higher potency of antimicrobial activity against the microorganism than other extracts. Though soxhlet extracts showed the presence of the most phytochemicals, these extracts exhibit lesser antimicrobial activity, which might be due to the hindrance of other compounds in the extracts [1].

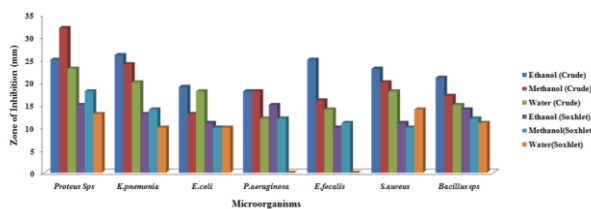
The antimicrobial activity of various plant extracts was due to the phytochemical constituents present in the extracts and concentrations of phytochemicals in the extracts were responsible for the effective antimicrobial activity.



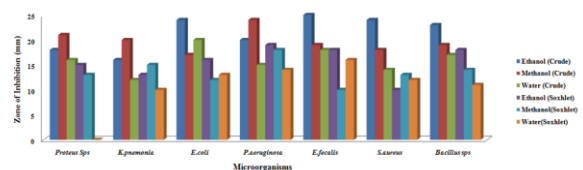
(a) Effect of *Acalypha indica* extracts on the Microorganisms



(b) Effect of *Azadirachta indica* extracts on the Microorganisms



(c) Effect of *Lawsonia inermis* extracts on the Microorganisms



(d) Effect of *Murraya koenigii* extracts on the Microorganisms

Fig. 1. Zone of Inhibition

In the present study, aqueous extracts showed lesser activity when compared to the alcoholic extracts and similar type of results were obtained by Dahiya Praveen et.al [10]. The lesser activity of aqueous extracts might be due to low concentration of antimicrobial compounds. Active compounds against microorganisms such as aromatic or saturated organic compounds are more often obtained through ethanol or methanol extraction [9].

The plants rich in tannins have antimicrobial potential due to the basic character that allows them to react with the protein to form stable water-soluble compound thereby killing the bacteria by directly damaging the cell membrane [13]. From the phytochemical analysis, the methanolic extracts showed the presence of tannins and higher total phenolic content. This proves the tannin-rich extracts are more active against the pathogens.

Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and it should not be surprising that they have been found in vitro to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to form a complex with extracellular and soluble proteins and it also forms a complex with bacterial cell walls [34].

Among the microorganisms gram-negative bacteria, *Proteus mirabilis* and *Enterobacter fecalis* were found more sensitive to the plant extracts. A similar type of observation was reported by Dhaiya Praveen et. al. [10] where the gram-negative organism was found to have more resistance.

#### 4. Conclusion

Methanolic and ethanolic extract of plants was found to reveal more number of phytochemicals when compared to the aqueous extract. Crude methanolic extract of *Murraya koenigii* has revealed the presence of all phytochemicals except tannins. In the quantitative phytochemical analysis of plant extracts shows, methanolic extract of *Acalypha indica* showed the higher total phenolic content and methanolic crude extract of *Murraya koenigii* was found to have a high content of Vitamin C (Ascorbic acid). From the results obtained by agar diffusion method, it was observed that the antimicrobial assay of the crude methanolic extract illustrated higher zone of inhibition compared to soxhlet extracts. The crude methanolic extract of *Lawsonia inermis* has showed higher zone of inhibition as compared to other extracts. Various active compounds present in the plant extracts increase the antimicrobial property of the plant extracts which were revealed qualitative and quantitative analysis. Further investigation of active compounds has to be done to isolate and characterize the bioactive compounds to develop new antimicrobial drugs.

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