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**Research Article**

# Antimicrobial Activity and Phytochemical Screening of Traditional Medicinal Plants

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## Abstract

In Nepal, traditional medicinal plants and herbs have long been relied upon for treating various diseases. This study was aimed to explore the antimicrobial properties of commonly used traditional medicinal plants. This study was conducted from December 2022 to June 2023 at the Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu. In this study, antimicrobial property of 12 plants were tested against four ATCC culturesof bacteria- *Staphylococcus aureus* (ATCC 6538P), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 8739) and *Pseudomonas aeruginosa* (ATCC 9027). The extracts were prepared using Soxhlet's apparatus with ethanol and distilled water as solvents. The ethanolic extract of *S. aromaticum* had the highest percentage yield The ethanolic extract of *O. sanctum* showed highest potential against *S. aureus* with a zone of inhibition of 21mm. The ethanolic extract of *S. aromaticum* demonstrated antimicrobial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, and *S. epidermidis*, with respective zones of inhibition of 18mm, 16mm, 18mm, and20mm. The distilled water extract of *A. marmelos* displayed the highest antimicrobial activity against *E. coli*, with a zone of inhibition of 18mm. Phytochemical screening revealed that both extracts of S. *aromaticum* had the highest presence of phytochemicals. The ethanol extract of *Z. armatum* exhibited the highest antibacterial activity, with a minimum inhibitory concentration (MIC) value of 2mg/ml against *S. aureus*. The study of the selected plants extracts confirmed their efficacy as natural antimicrobials against the tested organisms, suggesting their potential for novel drug development in treating infectious diseases.

**Keywords:** Plant extract; Phytochemical; Antimicrobial activity; Minimum Inhibition Concentration

## Introduction

Over 60% of the world's population, and about 80% in developing nations, stillrely exclusively on medicinal plants for their medical needs, making traditional medicine the preferred primary healthcare system in many communities. (Mintah, 2019). All the hilly, Himalayan, and Terai regions of Nepal contain medicinal plants. About 7000 species of flowering plants and 4000 species of non-flowering plants among which about 1500-1800 species are being used by local communities to treat various ailments, and more than 100 plants/plant parts areannually traded in and out of Nepal as medicinal and aromatic Plants (Gurung & Pyakurel, 2017).

Plant-based antimicrobials have a huge medicinal potential. They effectively treat infectious infections while also minimizing the number of adverse effects frequently connected to synthetic antimicrobials. Some of the drugs obtained from plants are aspirin, atropine, artemisinin, colchicine, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinine, quinidine, reserpine, taxol, tubocurarine, vincristine, and vinblastine (Garg *et al*., 2020). The variety of secondary metabolites found in plants is often what gives plant materials their helpful therapeutic effects.

Plants comprise various kinds of chemical constituents known as phytoconstituents (Mercy *et al*., 2017). They are classified into curative (or nutritive) such as alkaloids, saponnins, tannins, flavonoids, phenols, glycosides, iso isoflavones, carotenoids, sulfides and non-curative (or non-nutritive) such as cyanide, oxalates, terpenes and terpenoids. These plant-derived compounds like flavonoids, quinine, terpenoids etc., carry out specific biological processes that enhance therapeutic activities like anti-carcinogenic, anti-mutagenic, anti- inflammatory and antioxidant properties (Batiha *et al*., 2020).

One of the greatest threats to public health in the world today is antimicrobial resistance (AMR). According to a recent estimate, 3.57 million of the 4.95 million deaths recorded globally in 2019 were due to antimicrobial resistance, which is more than many other well-known causes of mortality, such as malariaand HIV/AIDS.

Many of Nepal's medicinal plants are on the verge of extinction as a result of the globalization of herbal medicine, unrestrained exploitative practices, and the absence of coordinated conservation efforts. However, here in Nepal, the isolation and purification of a particular bioactive compound from the herbs is still not yet fully explored. Therefore, this study will help us gaina better understanding of how they are used and how they affect both common microorganisms and drug-resistant ones. The main objective of this study is to prepare plant extracts using different solvents and screening the phytochemical properties along with determination of their antimicrobial activities, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC).

## Materials and Methods

Organisms used in the study were *Escherichia coli* (ATCC 8739), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus epidermidis* (ATCC 12228), and *Staphylococcus aureus* (ATCC 6538P) which were received from Nepal Public Health Laboratory, Teku (NPHL).

Plants used in the study were *Psidium guajava* (Guava leaves), *Allium cepa* (onion bulb), *Syzygium aromaticum* (Clove), *Nyctanthes arbortristis* (Parijat), *Ficus religiosa* (Peepal leaves), *Mimosa pudica* (Touch me not plant), *Aegle marmelos* (bel leaves), *Cinnamomum tamala* (Indian bay leaves) *Cinnamomum zeylanicum* (cinnamon), *Artemisia vulgaris* (mugwort leaves), *Ocimum sanctum* (holy basil leaves), and *Zanthoxylum bungeanum* (Timur)

The research was conducted from December 2022 to July 2023 at Department of Microbiology, St. Xavier's College, Maitighar, Kathmandu. A laboratory based descriptive cross-sectional study. The sampling technique used was purposive sampling, where samples were collected considering the purpose of study.

Dried plants were collected and washed with distilled water and disinfected by70% ethanol. Washed plant samples were then oven dried at 50℃ for a day.

Soxhlet's extraction method: Dried plants were grinded using a grinder into finepowder, twenty-five grams of plant powder was kept in the thimble made up ofblotting paper and inserted into the extraction chamber of the Soxhlet's apparatus. Following this, the solvents (distilled water and 80% ethanol) were added in the Soxhlet's round bottom flask respectively, which is then attached to Soxhlet extraction chamber and condenser on an isomantle, where the heat is applied and Soxhlet is left to run for about 5 cycles (Redfern, *et al*., 2014).

***Identification and preparation of Microbial culture***

The test bacteria were inoculated in Nutrient Agar and was incubated at 37°C for 24 hours and was gram stained and were subjected to various biochemical tests in order to identify it. The test organisms were maintained at 4°C. Active cultures for each bacterial species were prepared by transferring a loop full of colony from the stock cultures to test tubes of nutrient broth and incubated at 37°C for 24 hours & the test tubes were then compared with 0.5 McFarland standard.

***Determination of Antibacterial activity***

Mueller Hinton Agar plates were used for the antibacterial activity of differentplant extracts at different concentrations. With the help of a sterile swab, MHA agar plates were swabbed with isolated organism and were left for about 10 minutes to let surface dry. Using sterile well borer 4 wells were made on agar plates. 30µl of extract of different concentration (500mg/ml, 200mg/ml,100mg/ml and 50mg/ml) and DMSO as negative control were loaded in each different well and left at room temperature for about 1 hour for absorption of extract. The plates were incubated at 37°C for 24 hours and zoneof inhibition were measured and recorded.

***Detection of Flavonoid***

1ml plant extract was treated with dilute HCl followed by the addition of dilute acid. Appearance of yellow color indicated the presence of flavonoid.

***Detection of Tannins***

2ml of plant extract was treated with few ml of dilute chloroform and acetic anhydride followed by the addition of concentrated sulphuric acid. Formation of green color indicated the presence of tannin.

***Detection of Glycosides***

2 ml of glacial acetic acid with one drop of 2% Ferric chloride solution was mixed with 5 ml of aqueous extract and 1 ml of concentrated sulphuric acid. Presence of brown ring at the interface of the layers indicated the presence of glycosides.

***Detection of Terpenoids by Salkowski's Test***

2 ml plant extract was treated with 2 ml of chloroform and few drops of sulphuric acid was added to the layer. A reddish-brown coloration at the interface indicated the presence of terpenoids.

***Detection of Saponins by Foam test***

5 ml plant extract was mixed with 20 ml of distilled water in a test tube and shaken vigorously. The formation of stable foam indicated the presence of saponin.

***Detection of Alkaloids using Marquis reagent***

To prepare Marquis reagent, 0.5 ml of formaldehyde was added to 5ml of concentrated sulphuric acid. To this reagent, 5 ml of plant extract was added, and appearance of green color indicated the presence of alkaloids.

***Detection of Anthraquinone***

2 ml plant extract was dissolved in DMSO. 0.5 ml of ammonia solution was added to the mixture. Then the mixture was shaken vigorously. Formation of reddish color indicated the presence of anthraquinone.

***Detection of Coumarin***

1ml plant extract was mixed with 1ml of 10% NaOH solution. Formation of yellow coloration indicated the presence of coumarin.

***Detection of Starch***

3-4 drops of iodine solution were added to 0.5ml of stock solution of plant extract. Formation of blue color indicated the presence of starch.

***Detection of Quinones***

1ml plant extract was added to 1 ml of concentrated sulphuric acid and formation of red color indicated the presence of quinones.

***Detection of phenols***

5ml of the extract was added to a few drops of 5% Ferric chloride and the formation of dark green color indicated the presence of phenols.

***Detection of steroids***

5ml of the extract was added to 1ml of chloroform and a few drops of sulfuric acid and acetic acid. The formation of greenish color indicated the presence ofsteroids

***Determination of Minimum Inhibitory Concentration (MIC)***

The stock solution of the plant extract was prepared by dissolving it in dimethylsulfoxide (DMSO), resulting in a concentration of 256 mg/ml. A series of tubes containing the Muller-Hinton broth and various concentrations of the plant extract were prepared through serial dilution. A tube containing 1 ml of broth and 1 ml of the stock solution was used to obtain a concentration of 128 mg/ml.Subsequently, 1 ml of this mixture was transferred to another tube containing 1ml of broth, resulting in a concentration of 64 mg/ml. This process was repeated until the desired number of 7 tubes and 2 dilutions were achieved. Each tube was inoculated with a standardized amount of the test microorganism, 1 ml of a bacterial suspension with 0.5 McFarland turbidity. Then, the tubes were incubated at a 37°C for 24 hours.

The tubes were examined for visible growth by observing the turbidity or clarity of the broth. The MIC (minimum inhibitory concentration) was determined as the lowest concentration of the plant extract that prevented the growth of the microorganism. To confirm the MIC, a small amount of broth from each tube was sub-cultured onto Nutrient agar plate and incubated for another 24 hours.

The MBC (minimum bactericidal concentration) was determined as the lowestconcentration of the plant extract that showed no growth on the agar plate (Fig. 5). Flowchart of Methodology is shown in Fig. 1.

## Result and Discussions

***Percentage Yield of Plant Extracts Using Soxhlet Extraction***

*Syzygium aromaticum* exhibited the highest ethanol-based extract yield (17.15%), while *Nyctanthes arbortristis* displayed the lowest (5.35%). In the case of distilled water as a solvent, the highest extract yield (14.10%) was observed with *Syzygium aromaticum*, whereas the lowest (2.50%) was recorded for *Ocimum sanctum* (Table 1 & Fig. 2).

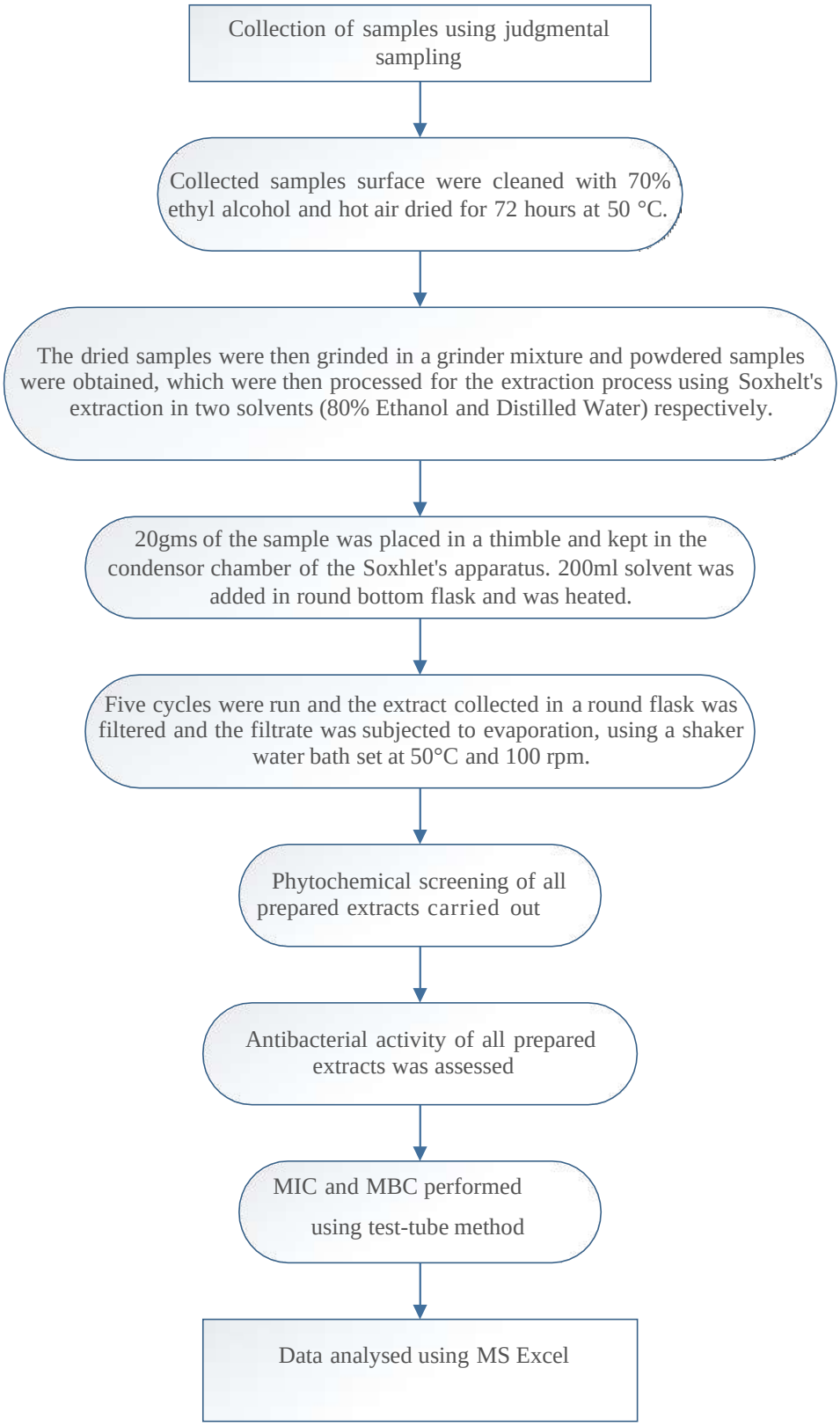


**Fig. 1**: Flowchart of Methodology

**Table 1**: Percentage yield for different extracts

| S. N. | Plant | Method of Extraction | Percentage Yield for Different Solvent | |
|---|---|---|---|---|
| | | | Ethanol | Distilled Water |
| 1 | *Syzygium aromaticum* | Soxhlet's Extraction | 17.15 | 14.10 |
| 2 | *Ocimum sanctum* | | 7.80 | 2.50 |
| 3 | *Cinnamomum tamala* | | 13.25 | 7.55 |
| 4 | *Nyctanthes arbor-tristis* | | 5.35 | 10.05 |
| 5 | *Mimosa pudica* | | 9.50 | 3.50 |
| 6 | *Aegle marmelos* | | 7.50 | 4.00 |
| 7 | *Psidium guajava* | | 6.80 | 6.00 |
| 8 | *Allium cepa* | | 7.25 | 6.75 |
| 9 | *Artemisia vulgaris* | | 8.60 | 8.25 |
| 10 | *Ficus religiosa* | | 7.30 | 4.15 |
| 11 | *Zanthoxylum armatum* | | 7.00 | 6.15 |
| 12 | *Cinnamomum verum* | | 8.15 | 3.20 |

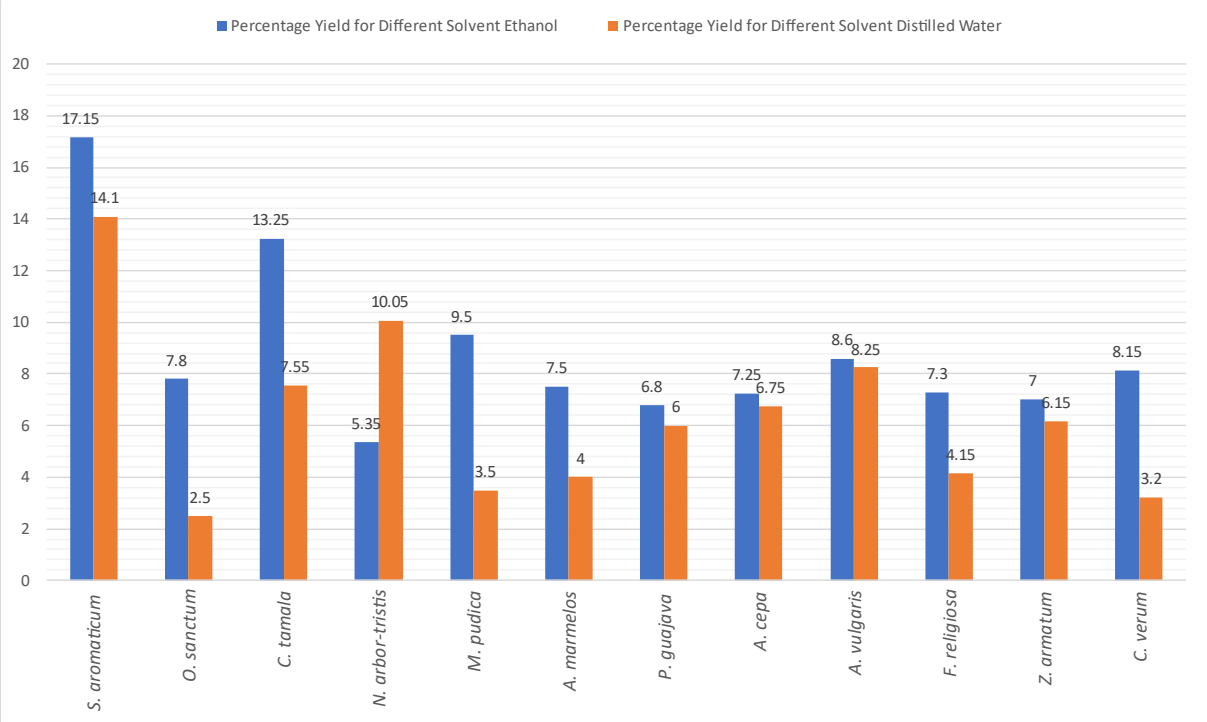


**Fig. 2:** Percentage yield for different plants for different solvents.

***Phytochemical screening of ethanolic and distilled water extracts***

Significant differences were found when different extracts were screened phytochemically in different solvents. A wide range of phytochemical groups, including flavonoids, tannins, glycosides, terpenoids, saponins, alkaloids, anthraquinones, coumarin, quinones, and steroids, were extracted more efficiently from ethanol than from distilled water. However, when it came to some plants, distilled water worked better than ethanol at removing flavonoids and glycosides (Table 2 & Table 3).

Table 2: Phytochemical analysis results for ethanol extraction

| S. N | Biological Name | Different Compounds | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 |
| 1 | *S. aromaticum* | + | + | + | + | - | - | + | - | - | + | - | - |
| 2 | *O. sanctum* | - | + | + | + | - | + | - | + | - | + | + | + |
| 3 | *C. tamala* | + | + | + | - | - | + | - | + | - | - | + | + |
| 4 | *A. vulgaris* | - | + | - | - | - | + | - | + | - | + | + | + |
| 5 | *A. cepa* | - | + | + | + | - | - | + | + | - | - | - | - |
| 6 | *P. guajava* | + | + | + | - | + | - | + | - | - | - | - | + |
| 7 | *N. arbor-tristis* | - | + | + | + | + | + | - | + | - | - | + | + |
| 8 | *M. pudica* | + | - | + | + | - | - | + | - | - | - | - | + |
| 9 | *A. marmelos* | + | + | + | + | + | - | - | + | - | - | + | + |
| 10 | *F. religiosa* | + | + | + | + | - | - | - | - | - | + | + | + |
| 11 | *C. verum* | + | + | - | + | + | + | - | + | - | - | + | + |
| 12 | *Z. armatum* | - | + | + | + | - | - | + | + | - | - | - | - |

(+) = Present; (-) = Absent
P1- Flavonoids, P2- Tannins, P3- Glycosides, P4- Terpenoids, P5- Saponins, P6- Alkaloids, P7- Anthraquinine, P8- Coumarin, P9- Starch, P10- Quinones, P11- Steroids, P12- Phenols

Table 3: Phytochemical analysis results for distilled water extraction

| S. N. | Biological Names | Different Compounds | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P1 | P2 | P3 | P4 | P5 | P6 | P7 | P8 | P9 | P10 | P11 | P12 |
| 1 | *S. aromaticum* | + | - | - | + | - | + | - | - | - | + | + | + |
| 2 | *O. sanctum* | + | + | + | + | - | - | - | + | + | + | - | + |
| 3 | *C. tamala* | + | + | - | + | + | + | - | + | - | - | + | + |
| 4 | *A. vulgaris* | + | + | + | + | - | - | - | - | - | - | + | + |
| 5 | *A. cepa* | + | - | - | - | - | - | - | - | - | - | - | + |
| 6 | *P. guajava* | + | - | + | - | - | - | - | - | - | - | - | + |
| 7 | *N. arbor-tristis* | + | + | + | + | - | - | + | - | - | + | - | + |
| 8 | *M. pudica* | - | - | - | + | - | - | + | - | - | - | + | + |
| 9 | *A. marmelos* | + | + | + | + | - | + | - | - | + | - | - | + |
| 10 | *F. religiosa* | + | + | + | + | + | - | - | - | - | + | + | + |
| 11 | *C. verum* | + | - | + | - | + | + | - | + | - | - | - | + |
| 12 | *Z. armatum* | + | + | + | - | + | - | + | + | - | - | + | - |

(+) = Present; (-) = Absent
P1- Flavonoids, P2- Tannins, P3- Glycosides, P4- Terpenoids, P5- Saponins, P6- Alkaloids, P7- Anthraquinine, P8- Coumarin, P9- Starch, P10- Quinones, P11- Steroids, P12- Phenols

***Antimicrobial activity***

In the ethanol solvent, *Mimosa pudica* exhibited the maximum inhibition zone against *E. coli* at 500mg/ml (19mm), while *Psidium guajava* showed the minimum inhibition zone at the same concentration (5mm). *Syzygium aromaticum* demonstrated the maximum inhibition zone against *E. coli* at 500mg/ml (18mm), while *Artemisia vulgaris* exhibited the minimum zone against *Staphylococcus aureus* (6mm). Against *P. aeruginosa*, *Syzygium aromaticum* displayed the maximum inhibition zone at 500mg/ml (16mm), *with Ficus religiosa* showing the minimum (6mm). In the case of *S. epidermidis*, *Ocimum sanctum* presented the maximum inhibition zone at 500mg/ml (21mm), while *Psidium guajava* and *Cinnamomum tamala* exhibited the minimum (9mm) (Table 4 & Table 5).

**Table 4**: Antimicrobial analysis result for ethanol extraction

| S. N. | Extract | Organisms | Inhibition (mm) for different concentrations (mg/ml) | | | | |
|---|---|---|---|---|---|---|---|
| | | | 500 | 200 | 100 | 50 | DMSO |
| 1 | *C. tamala* | *S. aureus* | 9 | 7 | 7 | 4 | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | 9 | - | - | - | - |
| 2 | *O. sanctum* | *S. aureus* | 10 | 7 | 7 | 6 | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | 10 | 9 | 7 | 6 | - |
| | | *S. epidermidis* | 21 | 19 | 18 | 8 | - |
| 3 | *S. aromaticum* | *S. aureus* | 18 | 19 | 9 | 8 | - |
| | | *P. aeruginosa* | 16 | 13 | 12 | 10 | - |
| | | *E. coli* | 18 | 16 | 11 | - | - |
| | | *S. epidermidis* | 20 | 19 | 19 | 16 | - |
| 4 | *A. marmelos* | *S. aureus* | 7 | 6 | 6 | 6 | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 5 | *N. arbor-tristis* | *S. aureus* | 16 | 14 | 14 | 8 | - |
| | | *P. aeruginosa* | 12 | 8 | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | 15 | 12 | - | - | - |
| 6 | *M. pudica* | *S. aureus* | 12 | 9 | 8 | 4 | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | 19 | 10 | - | - | - |
| | | *S. epidermidis* | 15 | 12 | 8 | 6 | - |
| 7 | *A. cepa* | *S. aureus* | 12 | 10 | 9 | - | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 8 | *P. guajava* | *S. aureus* | 9 | 7 | 6 | - | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | 5 | 3 | 2 | - | - |
| | | *S. epidermidis* | 9 | 6 | 4 | - | - |
| 9 | *A. vulgaris* | *S. aureus* | 6 | 5 | - | - | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 10 | *Z. armatum* | *S. aureus* | 10 | 8 | 6 | 4 | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | 11 | 8.5 | 8 | - | - |
| 11 | *F. religiosa* | *S. aureus* | 13 | 9 | 8 | 7 | - |
| | | *P. aeruginosa* | 6 | - | - | - | - |
| | | *E. coli* | 8 | 5 | - | - | - |
| | | *S. epidermidis* | 16 | 13 | 10 | 8 | - |
| 12 | *C. verum* | *S. aureus* | 17 | 12 | 7 | 6 | - |
| | | *P. aeruginosa* | 12 | 6 | - | - | - |
| | | *E. coli* | 10 | 7 | - | - | - |
| | | *S. epidermidis* | 10 | 7 | 6 | 5 | - |

**Table 5**: Antimicrobial analysis results for distilled water extraction

| S. N. | Extract | Organisms | Inhibition (mm) for different concentrations(mg/ml) | | | | |
|---|---|---|---|---|---|---|---|
| | | | **500** | **200** | **100** | **50** | **DMSO** |
| 1 | *C. tamala* | *S. aureus* | 13 | 12 | 9 | 6 | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | 7 | - | - | - | - |
| | | *S. epidermidis* | 19 | 12 | 11 | 9 | - |
| 2 | *O. sanctum* | *S. aureus* | - | - | - | - | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 3 | *S. aromaticum* | *S. aureus* | 12 | 10 | 6 | - | - |
| | | *P. aeruginosa* | 16 | 8 | - | - | - |
| | | *E. coli* | 12 | - | - | - | - |
| | | *S. epidermidis* | 10 | - | - | - | - |
| 4 | *A. marmelos* | *S. aureus* | 14 | 13 | 12 | 12 | - |
| | | *P. aeruginosa* | 18 | 13 | 13 | 12 | - |
| | | *E. coli* | 12 | 13 | 8 | 8 | - |
| | | *S. epidermidis* | 13 | 12 | 10 | 8 | - |
| 5 | *N. arbor-tristis* | *S. aureus* | 14 | 13 | 12 | 8 | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 6 | *M. pudica* | *S. aureus* | 19 | 14 | 11 | 6 | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | 14 | 12 | 9 | 6 | - |
| 7 | *A. cepa* | *S. aureus* | 6 | 4 | 2 | - | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 8 | *P. guajava* | *S. aureus* | 4 | 3 | - | - | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 9 | *A. vulgaris* | *S. aureus* | 7 | 6 | 4 | - | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 10 | *Z. armatum* | *S. aureus* | - | - | - | - | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 11 | *F. religiosa* | *S. aureus* | - | - | - | - | - |
| | | *P. aeruginosa* | - | - | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | - | - | - | - | - |
| 12 | *C. verum* | *S. aureus* | 12 | 10 | 8 | 7 | - |
| | | *P. aeruginosa* | 7 | 6 | - | - | - |
| | | *E. coli* | - | - | - | - | - |
| | | *S. epidermidis* | 12 | 10 | 10 | 6 | - |

***Minimum Inhibitory Concentration (MIC) of plant extract***

*S. aureus* was resistant to the antibacterial effects of all produced extracts. In solvent ethanol, *A. marmelos* extract was able to show activity only against *S. aureus* while in solvent distilled water it showed significant activity against all the test organism. The highest zone of inhibition against *E. coli* was exhibited by distilled water extract of *A. marmelos* which was 18mm. Both the ethanolic and distilled water extracts of *Allium cepa* and *Artemisa vulgaris* exhibited zone of inhibition against *S. aureus* only (Fig. 4).

***Minimum Inhibitory Concentration (MIC) of plant extract on S. aureus and E. coli***

The antimicrobial activity for *S. aureus* ranged from inhibiting at 128mg/ml with *A. marmelos* plant extract to completely inhibiting with *Z. armatum* plant extract. The antimicrobial property of the plant extracts against *S. aureus* can be ranked as the pattern: *Z. armatum* > *S. aromaticum* = *C. verum* > *M. pudica* > *P. guajava* = *O. sanctum* > *F. religiosa* = *A. marmelos.*

The antimicrobial activity for *E. coli* was exhibited only by *C. tamala* extract with the MIC value of to be 64 gm/ml.

**Fig. 3**: Phytochemical testing of Ethanolic extract of *Syzygium aromaticum*

**Fig. 4**: Zone of inhibition shown by *A. vulgaris* against *P. aeruginosa*

**Fig. 5**: MBC of *S. aureus*

Plants have been used for therapeutic reasons in both Western and Eastern cultures for over 60.000 years (GosseI-William *et al.*, 2012). Plant-derived antimicrobials have significant therapeutic potential. They are helpful in the treatment of infectious disorders while avoiding many of the negative side effects commonly associated with synthetic antimicrobials. Plant materials favorable therapeutic effects are usually due to the combination of secondary compound found in the plant (Joshi *et al.*,2008).

The average percentage yield of extract using ethanol as solvent was 8.54%, while the average percentage yield of extract using distilled water as solvent was 6.26%. This suggests that ethanol is a more effective solvent than distilled water for extracting compounds from these plants. The standard deviation of the percentage yield of extract using ethanol as solvent was 3.42%, while the standard deviation of the percentage yield of extract using distilled water as solvent was 3.14%. This indicates that there is more variation in the percentage yield of extract using ethanol as a solvent than using distilled water as a solvent.

As demonstrated by the examination of several types of phytochemicals (Fig. 3), including flavonoids, tannins, glycosides, terpenoids, saponins, alkaloids, anthraquinones, coumarin, quinones, and steroids, the choice of solvent has a major impact on the phytochemical content of plant extracts. Except in some cases where distilled water performs better in extracting flavonoids and glycosides, ethanol is more effective than distilled water. These findings also resemble findings by Plaskova & Mlcek, (2023). Phytochemical profiles of plants show diversity; some, such as *S. aromaticum*, *C. tamala*, *N. arbortristis*, and *F. religiosa*, show substantial diversity in both ethanol and distilled water extracts, suggesting a wide range of possible biological activities. *A. cepa* and *P. guajava*, on the other hand, exhibit minimal variability, pointing to a more limited therapeutic range. Variations in the presence of a substance are caused by a number of variables, including solubility, extraction effectiveness, condition sensitivity, and trace quantities. Tailoring extraction protocols and analytical techniques considering these factors is essential for optimal recovery and identification of specific phytochemicals in plant extracts (Khelurkar, *et al*., 2019).

Gram-positive bacteria exhibited higher susceptibility to plant extracts than gram-negative bacteria, attributed to differences in cell wall composition (Nikaido, 2003). The agar well approach demonstrated greater inhibitory effects compared to the disk diffusion method, possibly due to higher drug release with the well technique (Bhalodia & Shukla, 2011; Eloff, 1998). Secondary metabolites of herbs, influenced by environmental factors, determine their antimicrobial actions (Dewick, 2002). The limited antimicrobial potency in distilled water extracts may be attributed to the cold percolation extraction method and the use of crude extracts. The choice of ethanol and distilled water as extraction solvents was influenced by their polarity, with ethanol's ability to extract both polar and non-polar compounds. Fresh extracts and alternative extraction methods could be explored for enhanced antimicrobial efficacy (Bhalodia & Shukla, 2011; Nascimento, *et al.*, 2000).

The MIC values corroborated the overall inhibitory trend observed in the screening, except for the *Z. armatum* extract, whose MIC value was the lowest, indicating superior antimicrobial activity compared to other plant extracts. A study on *Z. armatum* extract reported the lowest MBC value of 0.78mg/mL for fruit extracts against MRSA, with MBC values of 1.56 mg/ml for fruits, seeds, and bark extracts against *S. aureus* (Phuyal *et al.*, 2020). In similar studies, methanol extracts of *Z. armatum* exhibited an MIC of 0.5 mg/mL against *S. aureus. S. aromaticum* bud oil demonstrated an MIC of 0.25 µL/mL against methicillin-resistant *S. aureus* (MRSA), and *C. verum* extracts had an MIC of 0.5 mg/mL against *S. aureus* (Kang *et al.*, 2011; Ak *et al.*, 2022; Oliveira *et al.*, 2022).

## Conflict of Interest

The authors declare that there is no conflict of interest with present publication.

## Author's Contribution

N. Mahat & M. Lawati designed the research plan; N. Mahat, N. Bhattarai performed experimental works & collected the required data. All authors jointly analysed the data; N. Mahat, N. Bhattarai & M. Lawati prepared the manuscript. All authors critically revised, finalized and approved the manuscript.

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