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# Screening of Phytochemicals and Antibacterial Property of Medicinal and Aromatic Plants of Nepal

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## Abstract

Medicinal and aromatic plants (MAPs) are gaining much attention as alternative therapy due to constant failure of chemotherapeutic agents. So, this study was conducted to screen the presence of different phytochemicals in MAPs and assess their antibacterial activity. For this, 4 different medicinal plant (*Ocimum sanctum*, *Acorus calamus*, *Artemisia vulgaris* and *Tinospora cordifolia*) were collected and extract was prepared. Phytochemical screening was done by chemical test. While antibacterial property was screened by agar well diffusion method against *Escherichia coli* and *Staphylococcus aureus.* The phytochemical tests revealed the presence of tannin and alkaloid in *O. sanctum* only; saponin in *A.calamus, T.cordifolia* and *A.vulgaris*; flavonoid in both *O.sanctum* and *A.vulgaris*; while phenol in *O.sanctum, A.calamus* and *A.vulgaris*. The antibacterial test revealed that *O. sanctum*, *A.calamus* and *A.vulgaris* inhibited the growth of both *E. coli* and *S. aureus* whereas *T.cordifolia* was the least inhibitory against both. Extracts of *O.sanctum, A.calamus* and *A.vulgaris* showed larger zone of inhibition (ZOI) against *S. aureus* than *E. coli*. Thus, it is concluded that selected medicinal plants possess one or more phytochemicals and can exhibit better antibacterial activity against *S. aureus* than *E. coli*, but a stratified analysis of each component is needed for a comprehensive knowledge.

***Keywords****: Medicinal plants, phytochemicals, antibacterial activity, Escherichia coli, Staphylococcus aureus*

## 1. Introduction

Treatment failure in clinical medicine at the expense of antimicrobial resistance bacteria is a leading public health concern of modern day (Murray *et al*, 2022). It is estimated by a study commissioned by the UK Government, that by the year 2050, death count by antimicrobial resistance (AMR) could reach 10 million people per year (O'Neill, 2014). Rapid development of multiple antibiotic resistance in microorganisms and different side effects of antibiotics in humans pose a challenge to treat infectious diseases for which medicinal plants can be used as an alternative medicine (Anand *et al*, 2019).

Medicinal plants are those plants that contain secondary metabolites that can be used for medicinal purposes or can be used as pioneer for discovery of novel homeopathic therapeutics (Penso, 1980). They have a long history in South east Asian countries and about 70-80% people worldwide still use traditional medicine to treat infections and meet their primary health care needs (WHO, 1999; Musyumi *et al*, 2008). The importance of medicinal plants for providing basic health needs of developing countries needs no emphasis.

A large number of plants of known medicinal and aromatic value grow wild in Nepal (Malla, 1991). The Medicinal and Aromatic Plants Database of Nepal (MAPDON) has estimated that among 350,000-500,000 medicinal plants found worldwide, 1,624 species are found in Nepal (Salmerón-Manzano *et al*, 2020; Shrestha *et al*, 2000). These medicinal plants include Tulasi, Tite-paati, Gurjo and Bojho along with many other plants (IUCN, 2000). Nepal has great prospect in herbal medicines if resources are utilized properly.

These phytochemicals are secondary metabolites of plants that protects the plant. These chemicals are found in different parts of plant like root, stem, leaves, fruit, seed, bark and flowers. Reportedly *O. sanctum* has phytochemicals like tannins, saponins, flavonoids, terpenoids, glycosides (Naik *et al*, 2015). Likewise, *A. calamus* contains glycosides, flavonoids, saponins, tannins, polyphenolic compounds, mucilage, volatile oil and essential oil (Chandra and Prasad, 2017). Similarly, A. *vulgaris* has also been reported to have vast array of phytochemicals like flavonoids, coumarins, phenolic acids, sterols, essential oils, polyacetylenes, carotenoids, artemisinin, vitamins, etc. (Ekiert *et al*, 2020). While, phytochemicals like alkaloids, furanolactone, diterpenoid lactones, cleodrane derivatives, glycosides, sesquiterpenoids and tinocordifolin, etc. are found in different parts of *T. cordifolia* (Tiwari *et al*, 2018). These phytochemicals have been studied and many of these apparently have antibacterial activity. For instance, antibacterial activity of *A. calamus* against AMR bacteria was reported by Devkota *et al* (1999) and Mahato et al (1998). Likewise, Farooq and Pathak (1998), Rathod *et al* (2012) and Juvatkar *et al* (2012) detected antibacterial activity of *O. sanctum*, *T. cordifolia* and other medicinal plants against pathogenic bacteria. Though several study on antibacterial activity of medicinal plants from Nepalese settings are readily available but continuity of such screening activity is necessary to understand the resistant profile of the evolving bacterial species. Evidently the infectious agents like bacteria and viruses are constantly developing resistance towards an array of chemical agents. Thus, continuous evaluation of antibacterial property of these medicinal plants against the pathogenic bacterial species especially resistant bacteria is necessary. Therefore, this study is screening the presence of different phytochemical in medicinal plants and detect any antibacterial activity presented by the plant extract.

## 2. Materials and methods

### 2.1. Collection of plant samples

Four different medicinal plants namely *Ocimum sanctum* (Tulasi) leaves, *Acorus calamus* (Bojho) root, *Artemisia vulgaris* (Tite-paati) leaves and *Tinospora cordifolia* (Gurjo) stem were collected from different parts of central Nepal based on their traditional medicinal uses. A total of 24 samples (6 samples of each medicinal plant) were used in this study.

### 2.2. Preparation of plant extracts (aqueous and ethanolic extract)

The medicinal plants were washed with water, shade-dried for 10-15 days at room temperature and grinded into powder using mortar and pestle. To prepare aqueous extract, powdered plant was soaked in distilled water in the ratio 1:20 (w/v) for 12-16 hours (Choudhary *et al*, 2013). The extract was then filtered and used for phytochemical tests. For ethanolic extract, the powdered plant was soaked in absolute alcohol in the ratio of 1:20 (w/v) and kept in a rotator for continuous shaking at room temperature and 35 rpm for 72 hours. Then, the solution was filtered through filter paper and kept in a water bath at 50ºC for 24 hours to get concentrated plant extract (Joshi *et al*, 2009). The obtained plant extract was suspended in 80% ethanol in a ratio 1:1 (alcohol: weight of powdered plant) and used to test antibacterial activity.

### 2.3. Phytochemical screening

Aqueous extract was used for phytochemical screening. The screening was done for tannins, saponins, flavonoids, phenols and alkaloids.

#### 2.3.1. Test for tannins

Twenty microliters (20µl) of 0.1% of ferric chloride was added to 1mL extract and observed for brownish green color (Saranya *et al*, 2019).

#### 2.3.2 Test for saponins

Twenty milliliters (20mL) distilled water was added to 1mL extract and shaken vigorously. Positive result was indicated by the formation of persistent froth (Saranya *et al*, 2019).

#### 2.3.3 Test for flavonoids

Twenty microliters (20µl) of 1% ammonia solution was added to 1mL extract and observed for yellow color (Choudhary *et al*, 2013).

#### 2.3.4 Test for phenols

Twenty microliters (20µl) of 10% ferric chloride solution was added to 1mL extract and observed for formation of black or green precipitate (Pandey and Tripathi, 2014).

#### 2.3.5 Test for alkaloids

Twenty microliters (20µl) of Dragendroff's reagent (Gram's Iodine) was added to 1mL extract and observed for formation of orange color (Saranya *et al*, 2019).

### 2.4. Preparation of test organisms

*Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were used as test organisms. Test organism was cultured in nutrient broth at 37ºC for 4 hours and the turbidity was matched with 0.5 Mc Farland before processing. (Aneja, 2018).

### 2.5. Antibacterial activity using agar well diffusion

Accurately 100 µl ethanolic extract was used to perform antibacterial activity by Agar Well Diffusion in Muller Hinton agar media plates (Othman *et al*, 2011) method and the susceptibility was noted in accordance to the criteria laid by Nascimento *et al* (2000). Ciprofloxacin (5mcg/liter) was used as positive control and ethanol was used as negative control.

### 2.6. Data analysis

Data were collected in observation sheet which was entered in MS Excel and then analyzed using SPSS 20.0 software.

## 3. Results and discussion

### 3.1. Phytochemical screening

Phytochemical screening revealed that at least one phytochemical was present in every medicinal plant tested. While for combination, both Tannin and phenol were present in one, saponin and alkaloid were present in three and flavonoid was present in two. Each phytochemical that tested positive in a specific plant was present in all the samples within the plant group.

**Table 1:** Presence of phytochemical in medicinal plants

| **Plant name** | **Number of samples** | **Phytochemical type** | | | | |
|---|---|---|---|---|---|---|
| | | Tannin | Saponin | Flavonoid | Alkaloid | Phenol |
| ***O. sanctum*** | 6 | + | - | + | + | + |
| ***A. calamus*** | 6 | - | + | - | + | - |
| ***A. vulgaris*** | 6 | - | + | + | + | - |
| ***T. cordifolia*** | 6 | - | + | - | - | - |

**Note:** (+) Present and (-) Absent

### 3.2. Antibacterial activity

The ethanolic extracts were investigated for evaluation of antibacterial activity using agar well diffusion method. Extracts of *O. sanctum*, *A. calamus* and *A. vulgaris* showed antibacterial activity against both *E. coli* and *S. aureus* while *T. cordifolia* was least effective against the test organisms. The range of ZoI produced by *O. sanctum* against *E. coli* and *S. aureus* was 8-10 mm and 19-23 mm respectively; by *A. calamus* against *E. coli* and *S. aureus* was 12-25 mm and 19-23 mm respectively; by *A. vulgaris* against *E. coli* and *S. aureus* was 11-13 mm and 24-29 mm respectively; by *T. cordifolia* against *E. coli* and *S. aureus* was 8-9 mm and 8-10 mm respectively. The results showed that the extracts were more effective against *S. aureus* than *E. coli*. The highest Zone of Inhibition was recorded to be 29 mm by *A. vulgaris* towards *S. aureus* followed by 25 mm which was by *A. calamus* against *E. coli*.

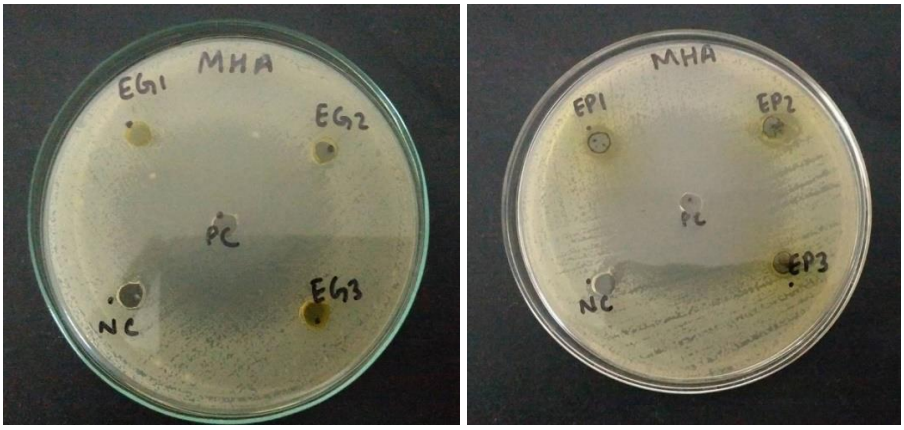


**Figure 1: ZoI diameter for *E. coli* by ethanolic extracts of *T. cordifolia* (left) and *A. vulgaris* (right)**

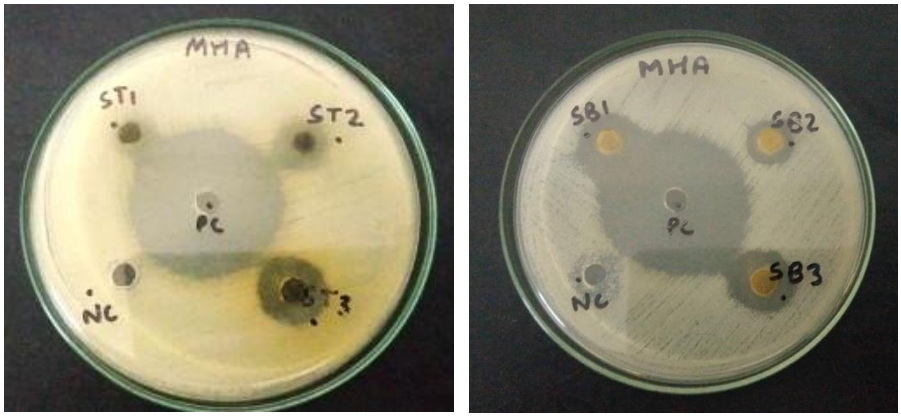


**Figure 2: ZoI diameter for *S. aureus* by ethanolic extracts of *O. snctum* (left) and *A. calamus* (right)**
**Note: PC- Positive control and NC- Negative control**

The plot below shows the diameter of ZoI against *E. coli* and *S. aureus* produced by ethanolic extracts of all 24 medicinal plant samples:

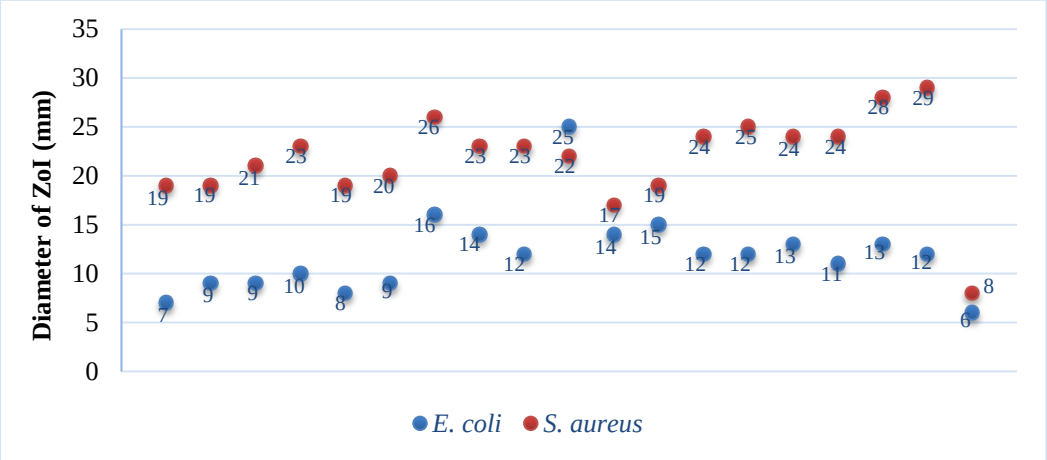


**Figure 3:** Scatter plot of diameter of ZoI of all 24 samples against *E. coli* and *S. aureus*

The descriptive statistics of ZoI of medicinal plants towards *E. coli* and *S. aureus* is given in the table below:

**Table 2:** Median of ZoI diameter produced by *E. coli* and *S. aureus for each sample type*

| **Ethanolic extract** | **Median** | |
|---|---|---|
| | ***E. coli* (mm)** | ***S. aureus* (mm)** |
| ***O. sanctum*** | 9 | 19.5 |
| ***A. calamus*** | 14.5 | 22.5 |
| ***A. vulgaris*** | 12 | 24.5 |
| ***T. cordifolia*** | 6 | 8 |

### 3.3 Discussion

Plants must exhibit antimicrobial properties in order to be considered as a medicinal plant. This study was conducted with the aim to screen the phytochemicals present in the selected medicinal plants and assess their antibacterial activity against common bacteria.

In this study, 5 phytochemicals were screened among which 4 phytochemicals namely tannin, saponin, alkaloid and phenol were present in *O. sanctum*. This result is similar to that of Biswas and Borah (2018) which reported the presence of phenol, flavonoid and alkaloids in aqueous extract of *O. sanctum* leaves. While, Saponin and phenol were present in *A. calamus* which is slightly similar to the results obtained by Nanda (2014) in which it was reported that alkaloid, flavonoid, tannin and phenolic were present but saponin was absent in *A. calamus* extract. Also, only saponin was present in *T. cordifolia* extract. Kavitha *et al* (2011) reported that the aqueous extract of *T. cordifolia* showed presence of saponin along with tannin, flavonoid and alkaloid. Saponin, flavonoid and phenol were present in *A. vulgaris* extracts. Kumar *et al* (2018) reported the presence of alkaloid, tannin, saponin and flavonoid in the aqueous extract of *A. vulgaris*.

According to the results, 83.33% and 100% ethanolic extracts of *Ocimum sanctum* (Tulasi) showed antibacterial activity against *E. coli* and *S. aureus* respectively. In a study by Mishra and Mishra, (2011), *Ocimum* extract inhibited the growth of both *Staphylococcus aureus* and *Escherichia coli*. Another study done by Shafi *et al*, (2018) also revealed antibacterial activity against *E. coli* (17.38±0.92 mm) and *S. aureus* (14.27±0.43 mm). Also, comparatively, this study shows that higher antibacterial activity of *O. sanctum* extracts was against *S. aureus* than *E. coli*. This result is in agreement with results of Mann *et al* (2000) but contradicting results can also be seen in study done by Mishra and Mishra (2011).

In this study, 100% ethanolic extract of *Acorus calamus* (Bojho) inhibited the growth of *E. coli* and *S. aureus* Study done by Susanah *et al*, 2018 resulted in optimum inhibition of pathogens like *E. coli* (concentration 8.0 to 16%) and *S. aureus* (concentration 12.0%). Also, Manikandan *et al* (2010) reported inhibition of *E. coli* (12 mm) and *S. aureus* (15 mm). Like *Ocimum* sample, *A. calamus* also revealed better antibacterial activity against *S. aureus* than *E. coli* which is similar to the results of study done by Manikandan *et al* (2010) but counters the findings of Susanah *et al* (2018). 100% ethanolic extract of *Artemisia vulgaris* (Tite-paati) inhibited the growth of test organism *E. coli* and *S. aureus*. This result is similar to that of Hrytsyk *et al* (2021) which reported to have inhibited the growth of *E. coli* (mean±S.D. ZoI 5.98±0.51mm) and Methicillin Susceptible *S. aureus* (mean±S.D. 4.23±0.61mm). Similar to other test MAPs sample, *A. vulgaris* extract also showed better antibacterial activity against *S. aureus* than *E. coli* which is contradictory to the results of study done by Hrytsyk *et al* (2021). 33.33% and 66.66% extracts of *Tinospora cordifolia* (Gurjo) had antibacterial activity towards *E. coli* and *S. aureus* respectively. While, Mishra *et al* (2014) reported *T. cordifolia* to have effective antibacterial activity against *S. aureus* but not *E. coli*. His study reported that the antibacterial activity was better against *S. aureus* than *E. coli* which is similar to the findings of our study.

## 4. Conclusion

The medicinal plants under study revealed the presence of phytochemicals and also showed the presence of antibacterial activity. Specifically, ethanolic extracts of *O. sanctum, A. calamus*

and *A. vulgaris* exhibited significant antibacterial activity against *E. coli* and *S. aureus* while *T. cordifolia* produced results that were below expectations. All of the extracts were more effective against *S. aureus* than *E. coli*. Undoubtedly the antibacterial activity of these medicinal plants is due to the presence of phytochemicals but further researches should be carried out to explore the potential medicinal application of these plants in various infections which may help in the discovery of novel drugs that can be used as alternative medicine to treat a wide variety of infectious diseases.

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**Conflict of interests**

The authors declare to have no conflict of interest.

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