Research Article

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# *Oroxylum indicum* leaf extracts for screening of antimicrobial properties and phytochemicals

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**Background:** *Oroxylum indicum* is mostly found in subcontinent of India. The stem, bark and roots of this plant have shown to have medicinal properties against inflammation, asthma, dysentery, cancer, fever, gastritis and respiratory disorders. Phytochemicals are compounds produced by plants which provide them defence against their competitors. They have been found to possess chemicals that are a potential source of medicines and products of industrial importance. In the current study, the extracts of leaves of *O. indicum* have been used to study the presence of phytochemical. Along with it, the leaf extracts have also been used to study their antimicrobial activity against *Pseudomonas aeruginosa* and *Bacillus subtilis*.

**Methods and findings:** The alcoholic extracts of leaves of *O. indicum* were tested for the presence of various phytochemicals, namely, terpenoids, phlobatannins, flavonoids, reducing sugars, phenols and tannins, carbohydrates, alkaloids and glycosides. The results showed that the leaves of *O. indicum* produce phlobatannins, flavonoids, phenols and tannins and glycosides. The antimicrobial activity of leaf extracts against *P. aeruginosa* and *B. subtilis* were observed as a clear zone of inhibition around the antimicrobial discs.

**Conclusion:** The current study reveals that the leaf extracts of *O. indicum* can be used as a potential source of phytochemicals and antimicrobial compounds for the production of novel drug molecules.

**Keywords: *Oroxylum indicum* · plant extracts · phytochemicals · antibacterial agents**

## Introduction

Plants synthesize a number of primary and secondary metabolites that have medical applications and can be used for therapeutic purpose. They are considered as a rich source of bioactive molecules which can be used for the development and synthesis of new drugs. Besides that these plants are involved in different human cultures around the whole world. Plants produce various chemical compounds for functioning biological activities. The mechanism of these bioactive compounds inside a human body is similar to the conventional pharmaceutical drugs with very less or no side effects. Till date, over 12,000 bioactive compounds have been reported [1]. The presence of many different biomolecules inside a single plant makes it difficult to consider taking the entire plant as a medicine. Medicinal plants hold a great significance in developing countries because of the fact that they are cheaper than the conventional medicines. Due to the increasing cost of modern medicines, the current research interest has shifted to the traditional herbal medicines with no or very less side effects. The use of medicinal plants for the treatment of diseases is not only restricted to developing countries but in few countries like Germany and France 70% of the doctors prescribe herbal medicines (Murray et al.). Many countries prefer plant-based medicines because of its non-toxic and cost effective nature. Also, the production of plant-based medicines does not require any harmful chemicals for its processing. Recently, thousands of plants are being used for their medicinal properties in various ethnic groups. Natural products derived from plants differ in their biological properties, structures and their mechanism of action. Phytochemicals are biochemicals produced by plants. The compounds like flavonoids,

polyphenols, and phenolic acids act as antioxidants that scavenge free radicals. India has one of the largest sources of medicinal plants which can be utilized as raw materials for the production of drugs and aromatic compounds. Other than these compounds, they are also used to produce food, nature dyes, insecticides and pesticides. Plants, such as, ginger, aloe, neem and tulsi have been used to cure many ailments and they have used as home remedies from ancient times.

## Phytochemistry of medicinal plants

Phytochemicals are not only nutritive plant chemicals but also are protective and contain properties of disease prevention and gives protection to human from numerous diseases. Studies on phytochemicals depict that plants which have antimicrobial activity composed of bioactive compounds e.g. saponnins, alkaloids, flavonoids and tannins. Flavonoids and alkaloids have been used as anticancer, antibacterial, antiviral agents. Plants derived disease control products have significant importance as they have properties like- wide acceptance, easily response to biological functions, environmental friendly, biocompatible, and nontoxic. Phytochemicals are naturally occurring plants chemicals present in medicinal plant parts that have defence mechanism and prevent from several diseases. Generally, two types of Phytochemicals are found. One is primary compounds and other is secondary compounds. Proteins, chlorophyll and sugar come under primary compound and alkaloids, terpenoid and phenolic compounds come under secondary compounds. Because of these secondary metabolites medicinal plants possess anti-fungal, anti-bacterial, anti-viral, anticancer, anti-analgesic, antidiuretic and anti-inflammatory activities. Screening and discovery of phytochemical compounds from medicinal plants are useful for production of novel drugs.

*Oroxylum indicum* (L) Vent. belongs to Bignoniaceae family. This plant is usually found in both Western ghats and Himalayas. This is very well known plant in India as it is used as an Indian form of medicine and it was also stated in Ayurveda as a Rasayana drugs.

## Geographical distribution of *Oroxylum indicum*

*Oroxylum indicum* is native to subcontinent of India. Generally it is found in some parts of Himalayas, Bhutan, Indo-china, South China and Malaysia Eco zones. It is also found in the forest of Manas National Park of Assam, India. It was also reported that *Oroxylum indicum* is found in Sri Lanka (Ceylon), Cambodia Yunnan, Taiwan, Sichuan, Guizhou, Guangxi, Guangdong, Fujian, Vietnam, Thailand, Philippines, Nepal, Myanmar, Malaysia and Laos [2,3].

## Medicinal uses of *Oroxylum indicum*

*Oroxylum indicum* is one of the most commonly used medicinal plants in Ayurveda medicines preparations such as Awaleha, Chyavanaprasa, Brahma Rasayana, Dhanawantara ghrita, Narayana taila, Dantyadyarista, Amartarista, Dasamula etc. [4]. Plants material such as bark, tannins, roots, and dyestuffs are also used for preparing medicines. *Oroxylum indicum* possess not only medicinal value but as well as economic value.

The roots and bark of this plant is bitter, pungent, acrid and astringent to the inflammation, asthma, leucoderma, dysentery, vomiting, intestinal worms, bronchitis, fevers, biliousness, useful in "vata", increases appetite, tonic, aphrodisiac, cooling, bowels and anal troubles. It was also reported that *Oroxylum indicum* is also used to treat rheumatism, diaphoretic, dysentery, diarrhoea and rheumatism [5,6]. Powder of *Oroxylum indicum* roots and bark with the sesame oil paste is used as a digestive tonic. The seeds of these plants are taken orally for curing hypertension and throat infections [7]. The fruits of this plant are anti-helminthic, stomachic, sweet, and acrid and it is effective in treating diseases of the bronchitis, piles, and diseases of the throat and heart. It is also as an expectorant, useful in leucoderma and helps in improving appetite leucoderma [8-10]. Leaves are used to treat to alleviate headaches, ulcers, treat as an enlarged spleen. Leaves are also reported as prescribed medicine for snake bite [9,10].

## Current status of research on *Oroxylum indicum*

Studies have been conducted on antioxidant activities of all the plants' parts but it was still not defined that which part possess highest antioxidant property. The antimicrobial, immunomodulatory and gastroprotective studies have been done with root bark and stem. Anti-inflammatory activity was also

performed on the leaves and stem bark, and antimutagenicity and antihepatotoxic studies have been done on leaves and fruits respectively. Still there is a lack of knowledge of what are the different phytochemical constituents present that are responsible for different biological activities.

## Materials and methodology

*Oroxylum indicum* have known to produce many phytochemicals, namely, alkaloids, flavonoids, phenols and tannins. In this present study, phytochemical and antimicrobial analysis of the leaf extract was performed.

### Collection of plant material

The plant, *Oroxylum indicum*, was collected locally from the Energy Park at Raipur [21.25°N 81.63°E] Chhattisgarh (India). This plant was used for its phytochemical analysis and antimicrobial test (**TABLE 1)**. The fresh and tender leaves of selected plants were used for extraction.

### Preparation of crude plant extract

Plant extract is a collection of crude mixtures extracted from different parts of plant. The tender leaves of selected plants were removed from the plant and washed under running tap water to remove dust from the surface of plant. Washed samples were chopped into small pieces and air dried. The dried plant parts were crushed with mortar pestle (**FIGURE 1A-C**). Finely crushed 200 g of plant sample was taken in a 1000 ml conical flask with 550 ml of absolute methanol. The conical flask was stored in dark for 10 days at 37°C with agitation after 24 hours. After 10 days the soaked sample was filtered using No.1 Whatman Filter Paper. The filtrate was concentrated at 40-50°C by using rotating evaporator until solid layer of plant extract was obtained. The solid plant extract was collected in 150 ml conical flask and stored at -4°C.

### Phytochemical tests

The plant extracts were screened for the presence of biologically active chemicals like glycosides, phenolic, tannins, flavonoids, saponins, sugars.

### Terpenoids test

A standard plant extract solution was prepared by adding 5 ml of absolute methanol to 5 mg of plant extract in a test tube. To the extract solution, 2 ml of

**Table 1. Phytochemical analysis of *Oroxylum indicum***

| S.No | Phytochemical | *Oroxylum indicum* |
|---|---|---|
| 1 | Terpenoid | Negative |
| 2 | Phlobatannin | Positive |
| 3 | Flavonoids | Positive |
| 4 | Reducing sugar | Negative |
| 5 | Phenols and Tannins | Positive |
| 6 | Iodine Test | Negative |
| 7 | Mayer's Reagent | Negative |
| 8 | Wagner's Reagent | Negative |
| 9 | Keller-Killiani Test | Positive |

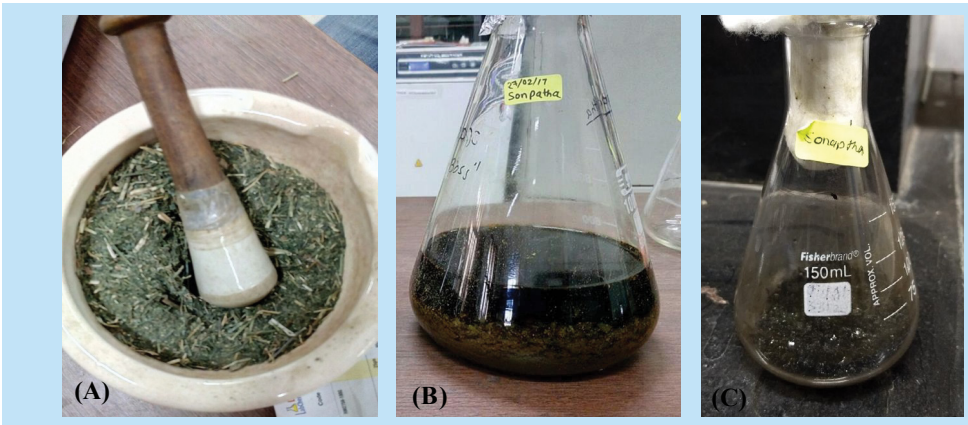


**Figure 1: Preparation of crude plant extract.** (A) Crushed leaves (B) Crushed leaves soaked in methanol (C) Concentrated plant extract

chloroform was added followed by 1 ml of concentrated $H_2SO_4$. The positive result was indicated by the formation of red colour solution as compared to no colour change of control.

### Phlobatannins test

The plant extract sample was prepared by adding 10 ml of distilled water to 20 mg of plant extract and in a test tube. 2 ml of diluted HCl was added to the sample and heated for 10 minutes at 80°C. Formation of brown colour precipitate solution indicated positive result with no precipitate in control.

### Flavonoids test

10 ml of distilled water was added to 20 mg of plant extract in a test tube. 5 ml of Ammonia solution (35% v/v) was added in the test tube followed by 1 ml of concentrated $H_2SO_4$. The positive result was indicated by the formation of red colour solution as compared to no colour change of control.

### Reducing sugars test

5 ml of distilled water was added to 25 mg of plant extract in a test tube followed by the addition of 1 ml of absolute methanol. Fehling's solution was prepared by mixing 1 ml of Fehling's A and 1 ml of Fehling's B solution and boiling it for 8-10 minutes and this solution was added to the extract solution. Formation of red colour precipitate indicated positive result when compared with control.

### Phenols and tannins test

10 ml of distilled water was added to 10 mg of plant extract in a test tube. 2 ml of 2% $FeCl_3$ solution was added to the solution. The formation of brown colour precipitate showed the presence of phenols tannins.

### Iodine test

10 mg of plant extract was mixed with 10 ml of distilled water in a test tube. 2 ml of Iodine solution was added to the test tube and observed for the formation of blue colour. The blue coloured solution indicated the presence of iodine.

### Mayer's test

10 mg of plant extract was added in a test tube containing 10 ml of distilled water. Mayer's reagent was prepared by mixing 50 ml of $HgCl_2$ solution (1.36 gm in 100 ml distilled water) and 50 ml of KI solution (5 gm in 100 ml distilled water). 5 ml Mayer's reagent was added to plant extract solution. Formation of red colour precipitate indicated presence of alkaloids.

### Wagner's test

10 ml of distilled water was added to 10 mg of plant extract in a test tube. Wagner's reagent was prepared by mixing 50 ml of Iodine solution (2 g in 100 ml distilled water) and 50 ml of KI solution (6 gm in 100 ml distilled water). The red colour precipitate indicated the presence of alkaloids in the sample.

### Keller-Killiani test

2 mg of plant extract was mixed with 10 ml of glacial acetic acid in a test tube with 2 drops of 2% $Fecl_3$ solution. 2 ml of concentrated $H_2SO_4$ was added to the solution. Formation of dark brown ring at the interface showed the presence of glycosides.

### Antimicrobial tests

*Pseudomonas aeruginosa* and *Bacillus subtilis* were used to test the antimicrobial activity of the plant extract. It was obtained from Department of Biotechnology, National Institute of Technology, Raipur (India).

### Inoculum preparation

Nutrient broth media was used for the preparation of inoculum. 10 ml of sterilized media was inoculated with 30 µl from microbial stock solution. The inoculum was incubated at 37°C in shaking incubator for 24 hours and then stored at 4°C for further use.

### Test methodology

For disc method, 0.1 ml of *P. aeruginosa* inoculum was spread onto nutrient agar plates. Antimicrobial discs were prepared by punching small disks from No 1 whatman filter paper. The discs were soaked in the plant extract solution (100 mg in 1 ml absolute methanol) for 10 minutes and placed on the centre of inoculated plates. The plates were incubated at 37°C for 24 hours.

For agar well diffusion methods, 0.1 ml of *B. subtilis* culture was spread onto nutrient agar plates. Well was punched into the agar. 50 µl of the crude leaf extract was added into the well and the plate was incubated at 37°C for 24 hours.

## Results

### Phytochemical tests

A lot of different tests were performed to check for various phytochemicals produced

by *O. indicum* which are considered as active constituents of medicinal plants. The phytochemical tests were performed on the leaf extracts of *O. indicum* which were dried using a rotary evaporator. The results of the phytochemical analysis showed that *O. indicum* produced four phytochemicals out of the nine compounds targeted. The test for terpenoids did not exhibit a red coloured solution which indicates that the leaf extract do not produce terpenoid. A brown coloured precipitate was observed in the phlobatannins test (**FIGURE 2A**). This shows that the plant extract produces phlobatannins. Phlobatannins possess antioxidant, analgesic, wound healing and anti-inflammatory properties and hence leaves of *O. indicum* can be used as a potential source of phlobatannins.

Flavonoids play a major role as antioxidants and in cell signalling pathways. They also have anti-allergic, anti-inflammatory, anti-cancer and anti-viral properties. The test for flavonoids resulted in the formation of a red coloured solution which indicates that the leaf extracts produce the phytochemical flavonoids (**FIGURE 2B**). The presence of reducing sugar is indicated by the formation of a red colour precipitate when the methanolic extract is made to react with Fehling's solution. In the present study, no red coloured precipitate was observed and hence reducing sugars were not produced (**FIGURE 3A**). A brown coloured precipitate is formed when phenols and tannins are mixed with ferric chloride solution (**FIGURE 3B**). The plant extracts showed a brown coloured precipitate after reacting with ferric chloride which confirms the presence of phenols and tannins in the extract of leaves.

The presence of carbohydrate, qualitatively, was tested by iodine's test. The presence of carbohydrate turns the solution to a blue colour. No change in colour was observed when the leaf extract of *O. indicum* was made to react with iodine solution. This shows that the extract does not contain carbohydrates (**FIGURE 4A**). The presence of alkaloids is detected by two tests- Mayer's test and Wagner's test. In both the tests, formation of red coloured precipitate indicates the presence of alkaloids. When leaf extracts of the plant were tested, no precipitate was observed both in Mayer's test and Wagner's test which indicated the absence of alkaloids in the plant extract (**FIGURES 4B and 5A**). Keller-killiani test was performed to detect the presence of cardiac steroidal glycosides in the leaf extracts. A dark brown ring was seen at the interface which confirmed the presence of glycosides (**FIGURE 5B**). Cardiac steroidal glycosides are an important

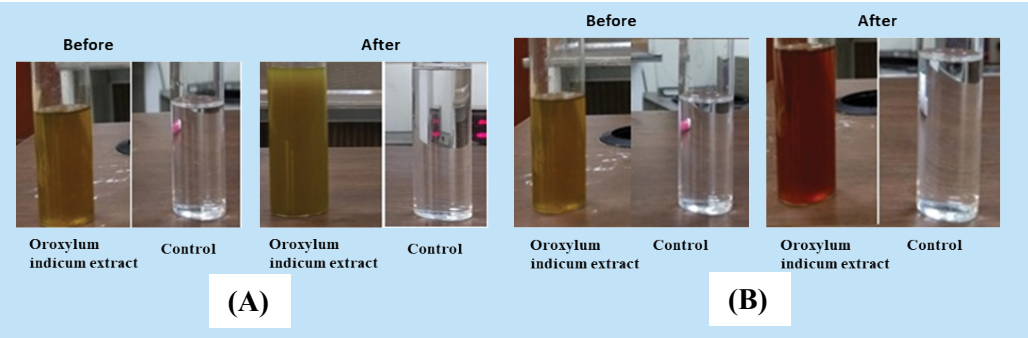


**Figure 2: The presence of phlobatannin.** (A) and flavonoids (B) in plant extracts of *O. indicum*

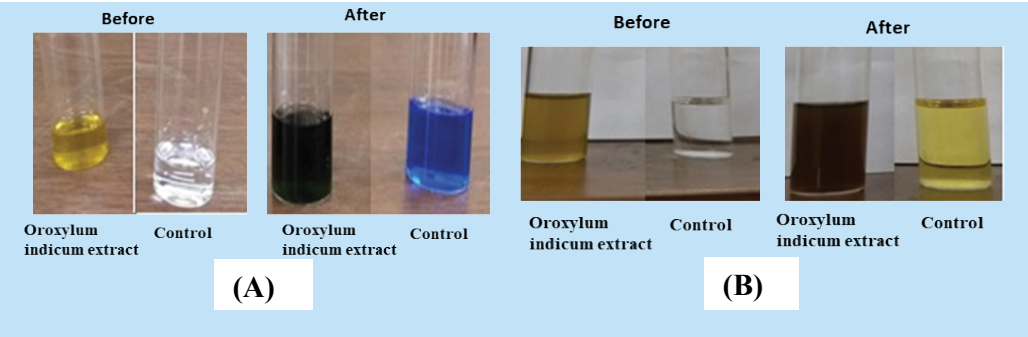


**Figure 3: The presence of reducing sugars.** (A) and phenols and tannins (B) in plant extracts of *O. indicum*

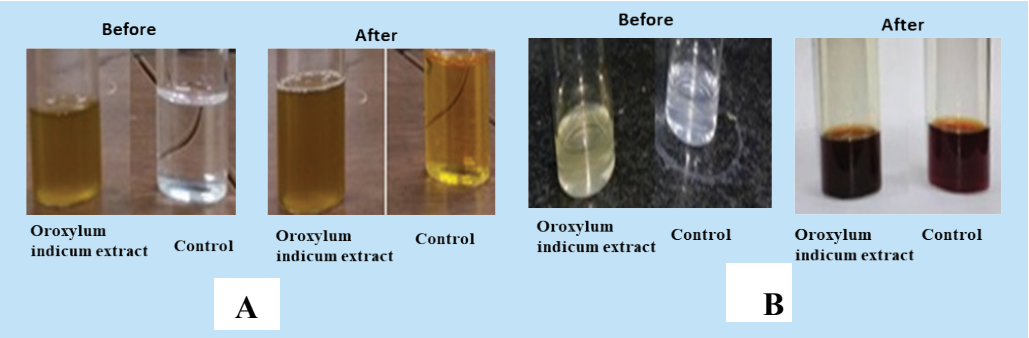


**Figure 4: The presence of carbohydrates.** (A) and alkaloids (B) in plant extracts of *O. indicum*

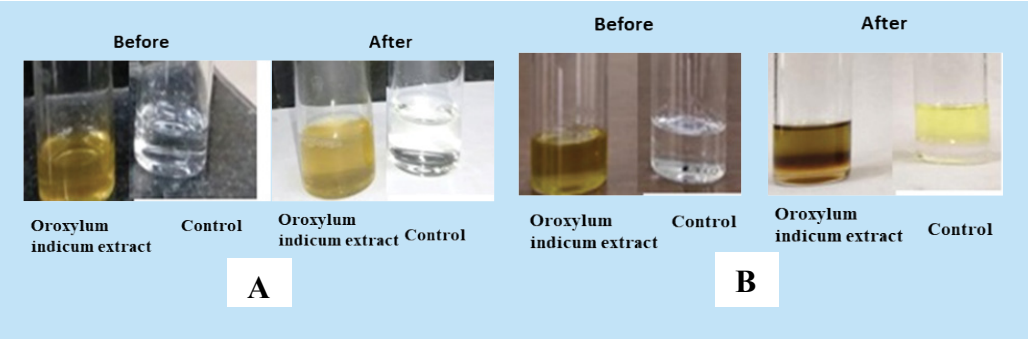


**Figure 5: The presence of alkaloids.** (A) and glycosides (B) in plant extracts of *O. indicum*

component to regulate heart rate and hence the plant extract can be used as a medication for cardiac regulation.

### Antimicrobial tests

The antibacterial activity of methanolic extracts of leaves of *Oroxylum indicum* was tested against *Pseudomonas aeruginosa*. The zone of inhibition was considered as the an indication of antimicrobial activity. When a disc containing antimicrobial compound is placed at the centre of a microbial culture a zone is formed which indicates that the antimicrobial compound inhibits the growth of the microorganism around it. Similarly, when plant extract is poured onto a well in agar plates, the extracts diffuse and act as antimicrobial agent killing the bacteria around the well. The size of the zone is measured from the edge of zone to the edge of disc. The zone of inhibition showed by the alcoholic extract of leaves of *O. indicum* against *P. aeruginosa* was found to be 0.6 cm (**FIGURE 6A and B** ). The well method for *B. subtilis* showed the zone of inhibition as 0.5 cm. This indicates that the leaf extracts of *O. indicum* produce some compounds that act against the growth of microorganisms (**TABLES 2 and 3**).

## Discussion

Various recent studies have reported the phytochemicals in bark and stem of *O. indicum* [2]. Lawania et al. showed the presence of alkaloids, flavonoids, glycosides, phenolic compounds and steroids in the alcoholic extracts of bark of *O. indicum* [11]. The extracts of seeds, stem bark and roots of *O. indicum* were used for the screening of the presence of various phytochemicals. The seed extracts showed the presence of alkaloids, flavonoids, glycosides, phenol, sterols, saponins, oils and fats. The root extracts showed positive results for alkaloids, fats, flavonoids, glycosides, lignins, phenols, saponins, sterols and tannins whereas the stem bark extracts were positive for fats and oils, phenols, saponins, sterols and tannins [12]. Das et al. demonstrated the presence of alkaloids, flavonoids, glycosides and tannins in the alcoholic extracts of barks of *O. indicum*. They also demonstrated the analgesic activity of the extract which indicates the development of novel analgesic molecules from the bark extract [13].

Ali et al. tested the stem bark and root extracts of *O. indicum* for its antibacterial activity. The extract exhibited antibacterial activities against *Bacillus subtilis*, *S. aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans*. The extracts also demonstrated anti-inflammatory activities [14]. The antibacterial activity of the stem extracts of *O. indicum* was tested against by

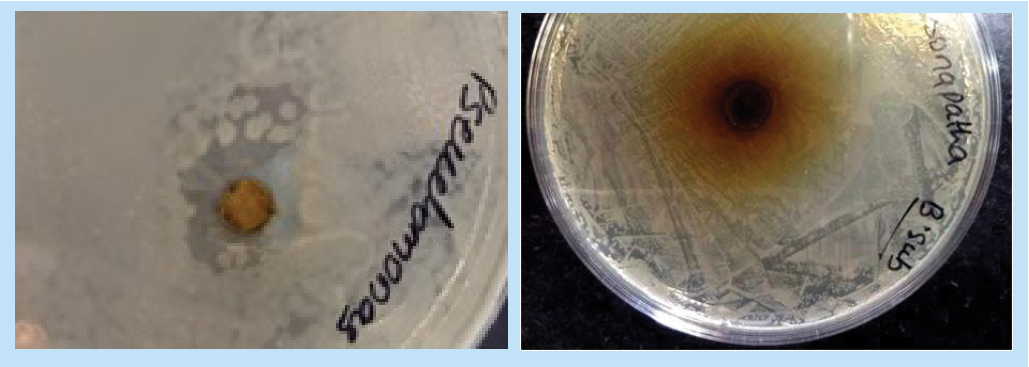

**Figure 6: Antimicrobial activity of leaf extracts of *O. indicum* against** (a) *P. aeruginosa* with disc method and (b) *B. subtilis* with agar well diffusion method

**Table 2. Zone of inhibition of *O. indicum* plant extract using antimicrobial disc method**

| Micro-organisms | Zone of Inhibition (radius in centimetres) |
|---|---|
| *Pseudomonas aeruginosa* | 0.7 |
| *Bacillus subtilis* | 0.5 |

**Table 3. Summary of the previously reported antimicrobial activities of *O. indicum* plant extract**

| S. No. | Part of the plant | Microorganisms | Reference |
|---|---|---|---|
| 1. | Stem bark and root | *Bacillus subtilis, S. aureus, Escherichia coli, Pseudomonas aeruginosa and Candida albicans* | 14 |
| 2. | Stem | *B. subtilis, E. coli, P. aeruginosa* | 15 |
| 3. | Stem | *S. aureus, Klebseilla sps, P. aeruginosa* | 16 |
| 4. | Root | *S. aureus and Proteus sps* | 16 |
| 5. | Stem bark | *E. coli, B. subtilis, B. cereus, K. pneumonia, P. aeruginosa S. aureus, Aspergillus fumigatus and Macrofomina phaeolina* | 7 |
| 6. | Stem bark | *B. subtilis, B. cereus, S. albus and S. aureus* | 18 |

against Gram positive and Gram negative bacterial strains [15]. The extracts showed potent antibacterial activity against *B. subtilis*, *E. coli* and *P. aeruginosa*. Radhika et al. reported the antimicrobial activity of *O. indicum*. They tested the alcohol extracts of root and stem against *E. coli*, *Klebseilla, Proteus, Pseudomonas* and *S. aureus*. The stem extract exhibited antimicrobial activity against *S. aureus*, *Klebsiella* sps., *P. aeruginosa* whereas the root extracts had antimicrobial activity against *S. aureus* and *Proteus* sp. [16]. A similar study was performed by Singh et al. 2012. They tested the antibacterial and antifungal activity of stem bark extracts of *O. indicum*. The alcoholic extract exhibited antibacterial activities against *E. coli*, *B. subtilis*, *B. cereus*, *K. pneumonia*, *P. aeruginosa* and *S. aureus* and antifungal activities against *Aspergillus fumigatus* and *Macrofomina phaeolina* [17]. Talari et al. reported the antibacterial activity of *O. indicum* stem bark extracts against *B. cereus*, *B. subtilis*, *S. albus* and *S. aureus* [18].

The studies till date have focussed on screening of phytochemicals and antimicrobial activity of *O. indicum* from the extracts of bark, stem, seeds or roots. None of the studies have targeted the leaves of *O. indicum* as a potent source of phytochemicals and antimicrobial agents. The present study demonstrated the presence of phlobatannins, flavonoids, phenols and tannins and glycosides as the phytochemicals in the alcoholic leaf extracts. These compounds have anti-allergic, anti-inflammatory, anti-cancer, anti-viral and anti-oxidant properties and can also be used as analgesics. The study also showed the antibacterial activity of the alcoholic leaf extract against *P. aeruginosa*, a Gram-negative microorganism and *B. subtilis*, a Gram-positive bacteria. The results indicate that the leaf extract of this plant can be used as a potential antibacterial agent against Gram positive and Gram negative bacterial and fungal species.

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