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# Phytochemical Analysis and Antimicrobial Activity of *Typhonium divaricatum*

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**Abstract**

Medicinal plants and natural products are scientifically interesting because of their potential application as phytodrugs. The phytochemical content of *Typhonium divaricatum* was studied qualitatively and quantitatively in this context. Only chloroform extract was found to contain alkaloid. Both aqueous and ethanol extracts contained flavanoid. All of the extracts tested were free of tannin, phenol, and saponin. Glycoside was found in nearly all of the extracts tested. The quantitative investigation found that the aqueous extract of *Typhonium divaricatum* contains high levels of steroids and carbohydrates, while the methanol extract contains high levels of steroids, carbohydrate, and glycoside. The chloroform extract contained a lot of steroids, terpenoids, and carbohydrates. The antibacterial activity of *Typhonium divaricatum* extracts in water, methanol, chloroform, ethanol, and butanol against *E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus mutans, Staphylococcus aureus*, and *Bacillus subtilis* was investigated. The highest inhibition was detected in butanol extract of *Typhonium divaricatum* against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, according to the screening results. Ethanol and butanol extracts of *Typhonium divaricatum* showed the greatest inhibitory zone against the fungus *Candida albicans*. As a result of these findings, *Typhonium divaricatum* should be further investigated as a source of natural compounds with adjuvant potential to boost antibiotic efficacy, hence reducing microbial antibiotic resistance.

**Keywords**: antimicrobial activity, carbohydrates, phytochemical phytodrugs and *Typhonium divaricatum*.

## Introduction

*Typhonium divaricatum* is often an ornamental plant with a pleasant odour and appearance, and this herb belongs to the Araceae family [1]. This plant's flowers are brown, deep maroon, and pink in colour [2]. Since *T. divaricatum* grows in both tropical and subtropical regions, this one-foot-tall, attractive plant is well-known in Asian nations, including Japan, Myanmar, and China [3]. Naturalized in Madagascar, Mauritius, Borneo, and the West Indies, among other places. Because of the beauty of the flowers, the plant is most commonly grown in gardens for ornamental purposes. Flowers are grown towards the base of this plant, unlike other plants [4] and the plant's proliferation arises from the tuber as a fresh birth of bulbs. *T.divaricatum* phytochemicals have anti-cancer properties, and this herb has been utilised as a traditional medicine for many years [5]. Antimicrobial properties are found in the bioactive component obtained from this plant [6]. The benefits of all kinds of species show excellent behaviours, but the *T. divaricatum* is highly irritant to skin and mucous membranes, so caution should be exercised during its preparation for use [7], as well as a wide variety of biological processes, such as cell to cell and host to pathogen interactions, and innate immune responses [3,8]. In this study, the efficacy of the plant extract against pathogenic and nonpathogenic bacteria, such as *Eschericia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis* and *Streptococcus mutans*, as well as fungi including *Aspergillus flavus, Aspergillus niger* and *Candida albicans*. To determine the bioactive compounds contained in the plant's crude extract, it was subjected to phytochemical screening using qualitative and quantitative tests using various solvents such as ethanol, methanol, chloroform, butanol, and aqueous.

## Materials and Methods

### Preparation of crude and solvent extract

The plant was collected and proved to be alive and well. To eliminate debris, the entire plant was cleaned with running tap water, then rinsed again with distilled water and dried in the shade. An electric blender was used to powder the dried plant. The Soxhlet extraction [9] method was used to extract bioactive components from a powdered sample of *Typhonium divaricatum* using solvents such as aqueous, methanol, chloroform, ethanol and butanol.

### Phytochemical screening

### Qualitative assay

The presence of phytochemicals in the whole plant extract was determined using a standard procedure to identify alkaloids [10], flavonoids [11], tanin, phenol, saponin and triterpenoids [12], carbohydrates [13], glycosides [14], steroids [15], fats and fixed oils [16].

**Quantitative assay**

To measure the amount of steroids [17], carbohydrate [18], glycoside [19], and terpenoids [20] in solvent extracts such as aqueous, methanol, and chloroform, a standard procedure was used.

**Antimicrobial Assay**

**Test organisms**

The antimicrobial test microorganisms that were utilised were *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus mutans, Staphylococcus aureus* and *Bacillus subtilis*. Fungi such as *Aspergillus flavus, Aspergillus niger* and *Candida albicans*, were acquired from the Microbial Type Culture Collection and Gene Bank (MTCC) in Chandigarh and the Nutrient Agar (NA) is used to maintain the bacterial strains and to maintain fungal strains Saboured dextrose agar (SDA) is used.

**Preparation of Nutrient Broth**

The disc diffusion method is used to determine the plant's effectiveness in five different solvents. The plate's pure culture was inoculated onto a Nutrient Agar plate. The inoculum was produced by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/l saline tube and incubated at 37°C for 24 hours. To get a bacterial suspension of 1.5108 CFU/ml, the cell density was adjusted to the 0.5 McFarland turbidity standards. Standard microorganisms were utilised in the antimicrobial test.

**Antibacterial activity**

The medium was dissolved, 38 g of Mueller-Hinton Agar Medium in 1000 ml of distilled water and autoclaved at 15 Lbs pressure for 15 minutes at 121°C at pH 7.3. The autoclaved media was cooled, well mixed, and put into petri plates (25 ml/plate). Bacteria were swabbed on the plates. Finally, the disc was placed on top of Mueller-Hinton Agar media, onto which the samples were poured, and the plates were incubated for 24 hours at 37°C. The size of the zone of inhibition was measured in millimetres and the zone of inhibition was checked around the disc and measured with a clear ruler in millimetres. The absence of zone inhibition was interpreted as the absence of activity [21, 22, 23].

**Antifungal Activity**

The agar disc diffusion [24] technique was used to evaluate antibiotic susceptibility. On the SDA agar plate, fungi strains were swabbed using sterile cotton swabs. Using sterile pipettes, up to 50µl of each concentration of the extract was put into the sterile disc. The disc was then placed on

the surface of SDA medium and the compound was allowed to diffuse for 5 minutes before the plates were incubated at 22°C for 48 hours. At the end of the incubation period, the zone of inhibition surrounding the disc was inspected and measured in millimeters with a clear ruler.

**Results and Discussion**

**Phytochemical Screening**

**Qualitative assay**

The existence and absence of phytocompounds in the plant *Typhonium divaricatum* were established in this study. Alkaloids were only found in chloroform extract, while flavonoids, fats, and fixed oils were found in aqueous and ethanolic extracts, respectively. Triterpenoids, glycoside, carbohydrate, and steroids were found in aqueous, methanol, and chloroform extracts, and a glycoside was also found in butanol extract. Table 1 clearly illustrated the presence and lack of phytocompounds. Experimental results shown from figures 1. Diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of valuable therapeutic key. Much of the protective effect of fruits and vegetables also has been attributed to the phytochemicals, which are the non-nutrient plant components. Different phytochemicals have been found to have a broad range of activities, which may help in protection against chronic diseases [25]. These phytochemicals present in the crude extracts may account for their various pharmacological activities. Alkaloids and flavonoids are the major phytochemicals found in *Typhonium flagelliforme* [26].

**Quantitative assay**

To determine the quantity of phytocompounds in aqueous, methanol, and chloroform extracts, such as steroids, carbohydrates, glycosides, and terpenoids. The quantity of steroids in aqueous, methanol, and chloroform was 440.5±0.5, 445.5±0.5, and 374.5±0.5 respectively, according to the data. Carbohydrate concentrations in aqueous, methanol, and chloroform extract were 343.5±1.5, 300.5±0.5, and 187.5±1.5 respectively. Glycoside quantities in aqueous, methanol, and chloroform were 19.5±0.5, 287±1, and 33.5±0.5 respectively. In aqueous, methanol, and chloroform extracts, the quantity of terpenoids was 38.5±0.5, 7.5±0.5, and 221.5±1.5 respectively. Table 2 indicated the quantity of phytocompounds and showed the high amount of steroids in aqueous, methanol and chloroform extract of *Typhonium flagelliforme*.

Medicinal plants have fascinated many researchers that subsequently lead to research publications highlighting plant extracts with wide range of secondary metabolites such as flavonoids, alkaloids, glycosides, quinones, terpenoids, terpenoids, tannins and saponins that

exhibit antimicrobial activities and disease control. The concentration of these bioactive compounds in each plant species varies based on the pathosystem and environmental conditions. A medicinal plant is in which one or more of its parts, contains functional phytochemical, which can be isolated and applied either for medicinal treatment or as a drug constituent [27]. The current results support findings of Shahriar *et al*. [28] who demonstrated the presence of various chemical groups including flavonoids, carbohydrate, phenols and alkaloids in plant *Typhonium trilobatum* (L.) Schott.

**Antimicrobial assay**

The selected plant is treated with bacteria such as *Eschericia coli, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis*, and *Streptococcus mutans*. The obtained result shows the absence of antibacterial efficacy in ethanol, methanol, chloroform, and aqueous. However, in *Klebsiella pneumoniae* with a 13 mm positive control, butanol has an inhibitory zone of 8mm and a clear zone of 7 mm in *Pseudomonas aeruginosa* with a 17 mm positive control. Table 3 shows the evaluated values with positive and negative controls. Experimental results shown from figures 2.

The antifungal activity of the five different solvents extracted were treated with *Aspergillus flavus, Aspergillus niger*, and *Candida albicans*. The obtained results are measured in millimeter (mm) range. In *Candida albicans*, the aqueous extract has a 15 mm inhibition zone, the ethanol extract has an 18mm inhibition zone, and the Butanol extract has a 12 mm inhibition zone. Methanol and butanol extracts create 9 mm of clear inhibition zone in *Aspergillus flavus*, whereas ethanol extract creates 10 mm of clear zone. There is no zone of inhibition in *Aspergillus niger* by any of the five distinct solvents. Table 4 shows the evaluated values with positive and negative controls. Experimental results shown from figures 3.

The antimicrobial activity in the plant may be due to the presence of high amount of bioactive compounds. Butanol extract showed highest antimicrobial activity because the presence of compound glycoside may be responsible for the antimicrobial activity. Flavonoid glycoside structural features such as the presence of an aromatic ring, the sugar moiety or the numbers of hydroxyl and methoxyl groups can significantly change membrane permeability and subsequent affinity to external and internal binding sites in the bacteria, thus influencing the compound's antimicrobial properties [29].

Ethanol extract inhibit the growth of fungi *A. flavus* and *C. albicans* and also aqueous extract inhibit the growth of fungus *C. albicans*. Ethanol and aqueous extract of *T. divaricatum*

contain phytochemicals such as flavonoid and fat & fixed oils may be responsible for the antifungal activity. Flavonoids possesses a mechanism by providing a source of stable free radical and also forms complex with nucleophilic amino acids in protein leading to the inactivation of the protein and loss of function, their potential antimicrobial effect is great as they probably target microbial cell of surface-exposed adhesins, cell wall polypeptides and membrane bound enzymes [30]. Therefore, they are well-known for their medicinal properties such as antiseptic, anti-carcinogenic, anti-inflammatory, analgesic, anesthetic and they are mainly used as natural additives in food and food products due to their antioxidant and antimicrobial properties.

The fungal cell wall consists of essential elements such as glucan, chitin and mannan for fungal survival. Phytochemicals in essential oils affect fungal cell wall maturation, septum formation and bud ring formation [31]. This leads to the thinning and distortion of the hyphal wall, thus causing the hyphal tip to be divided into bud-like structure. The severity of damage can be up to the level where the cytoplasm leakage inhibits DNA, RNA, protein and peptidoglycan biosynthesis and, lastly, inhibits the ergosterol biosynthesis [32].The antifungal activity of essential oil towards mitochondrial damage was comprehended to be the role of terpenoids that give rise to an altered level of reactive oxygen species and ATP generation [33].

Antimicrobial activity of plant *T. divaricatum*, the methanolic extract only inhibit the fungus *A. flavus.* The phytoconstituents including triterpenoids, glycosides and steroids present in the ethanol extract; these compounds responsible for the antifungal activity.

Terpenoids are for dissolution of the cell wall of microorganisms by weakening the membranous tissue [34]. Terpenoids exhibit antifungal, antimicrobial, antiviral, antiparasitic, antispasmodic, antiallergenic, anti-inflammatory, immunomodulatory and antihyperglycemic properties [35]. According to Mohan et al. [36] hexane extract of *Typhonium flagelliforme* had antibacterial activity against *P. aeruginosa*. Phenylpropanoidglycsides, sterols and cerebroside which has antihepatic activity were reported from the root of the plant *T. flagelliforme* [37]. Roy et al. [38] reported methanol, chloroform and ethyl acetate extract of *Typhonium trilobatum* plant has antimicrobial activity against six different microorganisms included *S. aureus, S. epidermis, E. coli, P. aeruginosa, C. albicans* and *A. niger*.Seeing all above results *Typhonium divaricatum* appears to be a promising plant demonstrating antibacterial activity that requires further investigation.

**Table 1: Qualitative Screening of *Typhonium divaricatum***

| Phytochemicals | Methanol | Aqueous | Chloroform | Ethanol | Butanol |
|---|---|---|---|---|---|
| **Alkaloid** | - | - | + | - | - |
| **Flavonoid** | - | + | - | + | - |
| **Tannin** | - | - | - | - | - |
| **Phenol** | - | - | - | - | - |
| **Saponin** | - | - | - | - | - |
| **Triterpenoid** | + | + | + | - | - |
| **Carbohydrate** | + | + | + | - | - |
| **Glycoside** | + | + | + | - | + |
| **Steroid** | + | + | + | - | - |
| **Fat and Fixed Oils** | - | + | - | + | - |

Whereas, (+) indicate the presence and (-) refers to absence of bioactive compounds.

**Table 2: Quantitative Analysis of *Typhonium divaricatum***

| Sl. No. | Tests | Aqueous (µg/g) | Methanol(µg/g) | Chloroform(µg/g) |
|---|---|---|---|---|
| 1 | Steroids | 440.5±0.5 | 445.5±0.5 | 374.5±0.5 |
| 2 | Carbohydrates | 343.5±1.5 | 300.5±0.5 | 187.5±1.5 |
| 3 | Glycosides | 19.5±0.5 | 287±1 | 33.5±0.5 |
| 4 | Terpenoids | 38.5±0.5 | 7.5±0.5 | 221.5±1.5 |

**Table 3: Antimicrobial activity of*Typhoniumdivaricatum***

| Solvents extract | *E. coli* | *K. pneumoniae* | *P. aeruginosa* | *S. aureus* | *S. mutans* | *B. subtilis* |
|---|---|---|---|---|---|---|
| Aqueous | NZ | NZ | NZ | NZ | NZ | NZ |
| Methanol | NZ | NZ | NZ | NZ | NZ | NZ |
| Chloroform | NZ | NZ | NZ | NZ | NZ | NZ |
| Ethanol | NZ | NZ | NZ | NZ | NZ | NZ |

| | | | | | | |
|---|---|---|---|---|---|---|
| Butanol | NZ | 8 mm | 7 mm | NZ | NZ | NZ |
| Positive control | 15 mm | 13 mm | 17 mm | 12 mm | 21 mm | 17 mm |
| Negative control | NZ | NZ | NZ | NZ | NZ | NZ |

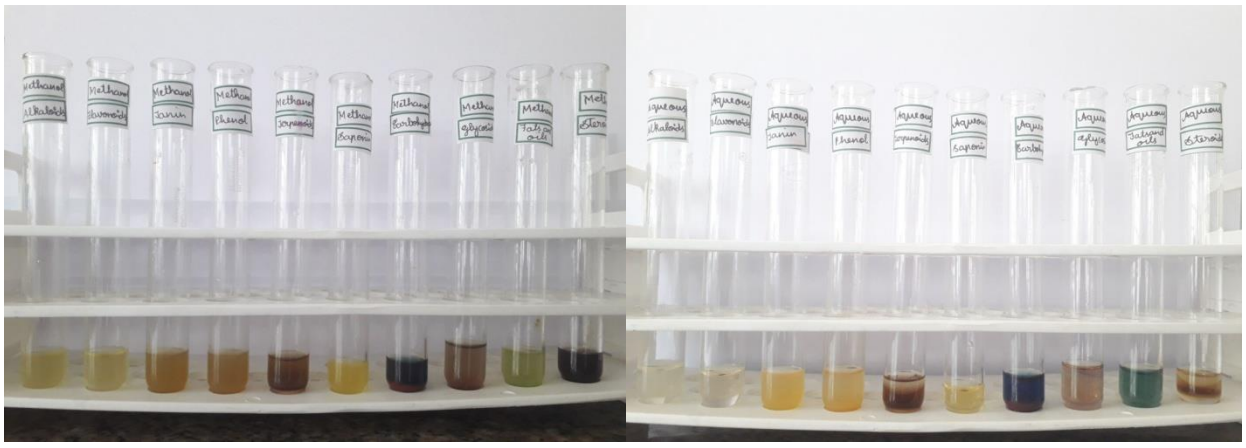
*NZ - no clear zone

**Table 4: Antifungal activity of *Typhoniumdivaricatum*.**

| **Solvents extract** | ***A. niger*** | ***A. flavus*** | ***C. albicans*** |
|---|---|---|---|
| Aqueous | NZ | NZ | 15 mm |
| Methanol | NZ | 9 mm | NZ |
| Chloroform | NZ | NZ | NZ |
| Ethanol | NZ | 10 mm | 18 mm |
| Butanol | NZ | 9 mm | 12 mm |
| Positive control | 15 mm | 26 mm | 20 mm |
| Negative control | NZ | NZ | NZ |

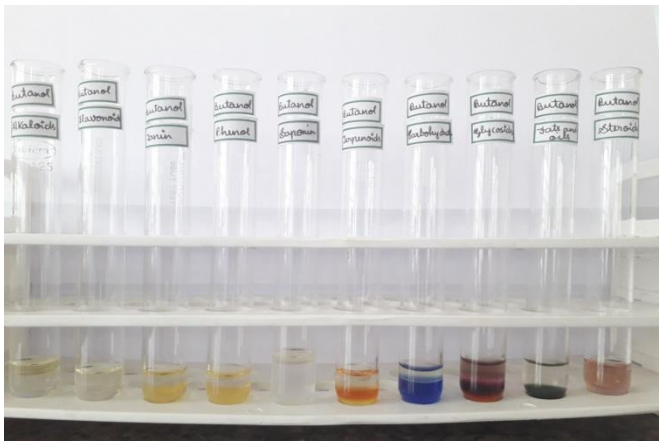
*NZ - no clear zone of inhibition.

**Figure 1. Qualitative analysis of *T. divaricatum***



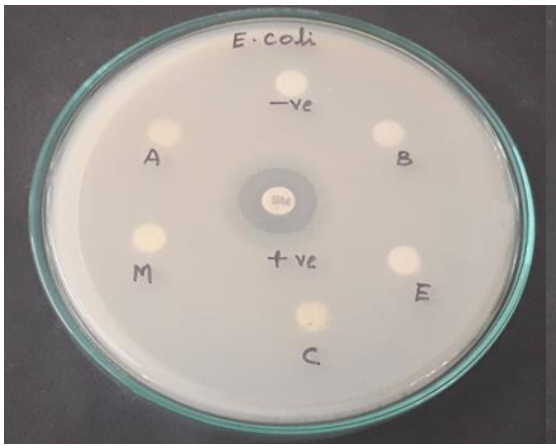
**Methanol** **Aqueous**

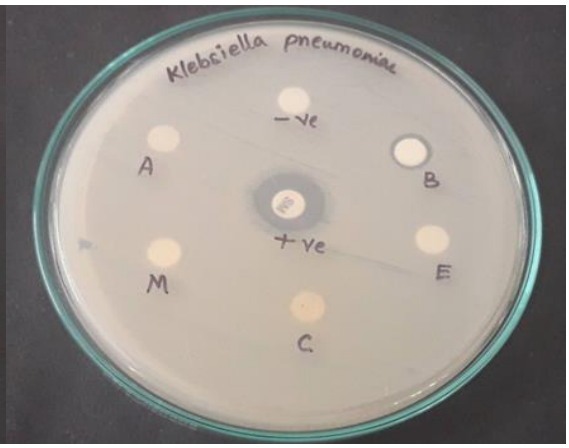
**Chloroform** **Ethanol**



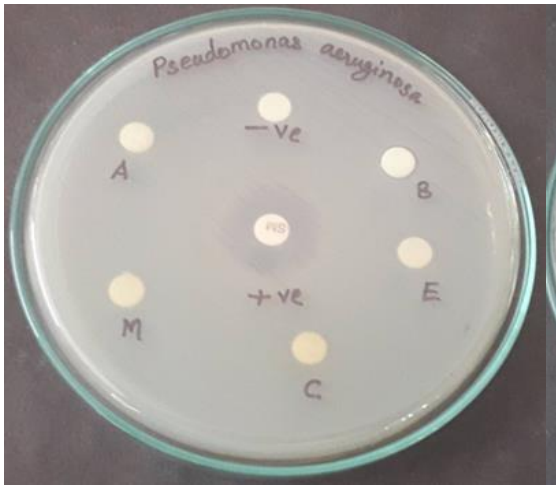
**Butanol**

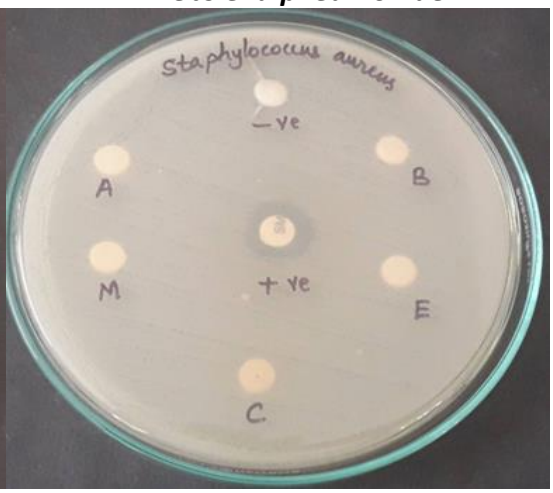
**Figure 2. Antibacterial Activity of *T. divaricatum***



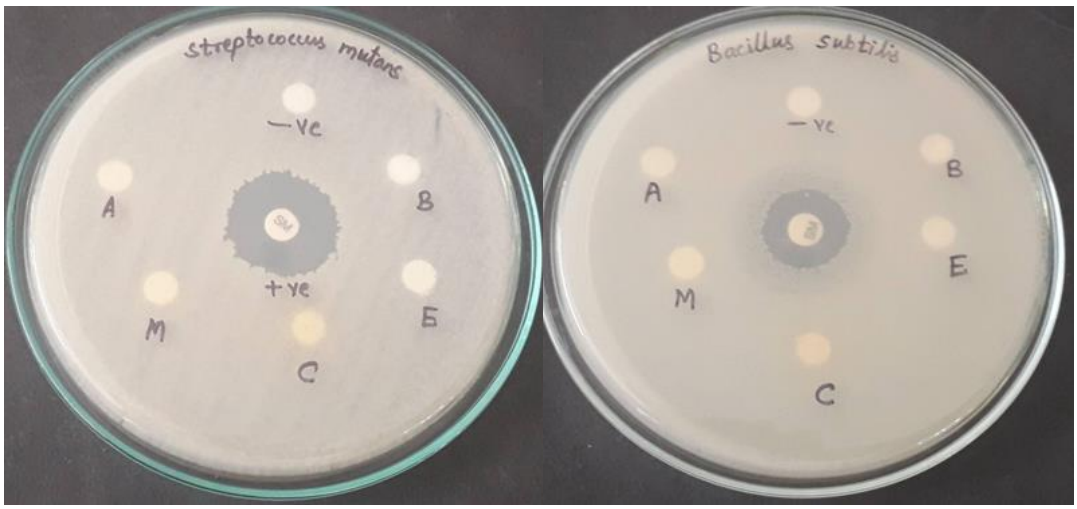




***Eschericia coli*** ***Klebsiella pneumoniae***



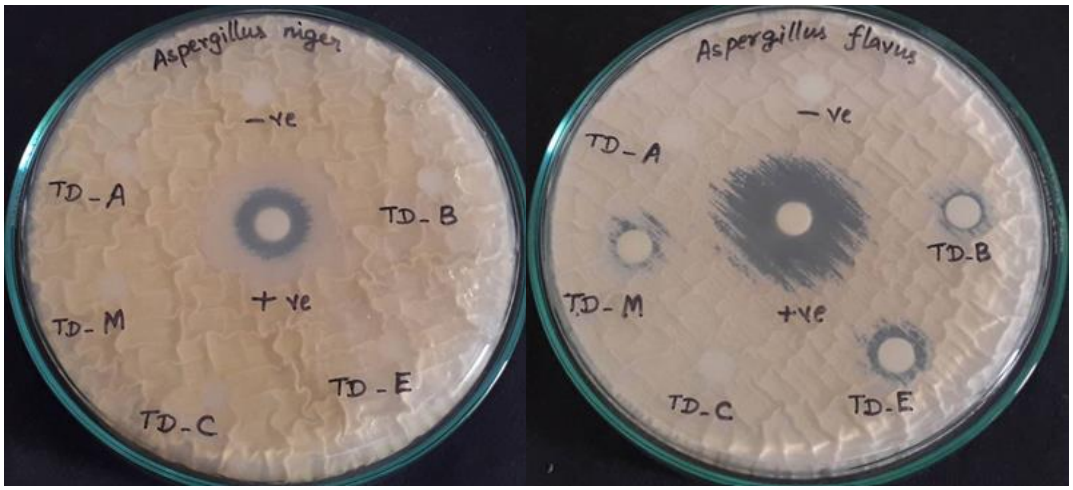




***Pseudomonas aeruginosaStaphylococcus aureus***

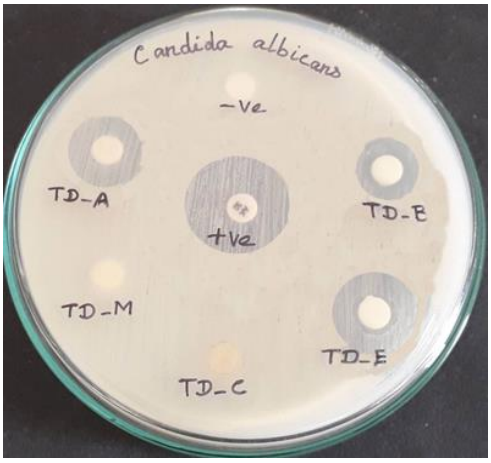



***Streptococcus mutansBacillus subtilis***

**Figure 3. Antifungal activity *T. divaricatum***




***Aspergillus nigerAspergillus flavus***




***Candida albicans***

**Conclusion**

In the traditional medical system, several plants are recognised to have positive medicinal benefits. As a result, plants are being investigated as a source of human illness management. Based on the fact that species from the same family (Araceae) contain several beneficiary compounds that assist in the treatment of a variety of ailments, the beneficiary of this specific species, *Typhonium divaricatum*, was evaluated and processed. To explore the bioactive compounds that might aid in the development of novel medicines in the pharmaceutical sector. Therapeutics require screening of various natural organic compounds and identifying active agents. The study's findings concluded that the specific species from the Araceae family also have many beneficial bioactive compounds under certain analysis. As a result, these species can be considered to be used in the production of novel medicines.

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