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**ORIGINAL ARTICLE** **OPEN ACCESS**

# *In Vitro* Antibacterial and antifungal activities from leaf extracts of *Tubiflora acaulis* Kuntze (Acanthaceae): An ethnomedicinal plant

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**ABSTRACT**
*Infections by microbes (viruses, bacteria, and fungi) and parasites can cause serious diseases in both humans and animals. The aim of the study is to assess the antimicrobial activity of leaves extract of Tubiflora acaulis Kuntze and to determine the zone of inhibition of extracts on some bacterial and fungal strains. The antimicrobial activity was determined in the extracts using agar well diffusion method. The antibacterial and antifungal activities of extracts (5, 25, 50, 100, 250 µg/ml) of Tubifloraacaulis Kuntze were tested against two Gram-negative Escherichia coli, Pseudomonas aeruginosa, Gram-positive Staphylococcus aureus, Streptococcus pyogenes, human pathogenic bacteria and three fungal strains Aspergillus niger, Aspergillus clavatus, Candida albicans. Zone of inhibition of extracts were compared with that of different standards like ampicillin, Chloramphenicol, Ciprofloxacin and Norfloxacin for antibacterial activity and Griseofulvin and Nystatin for antifungal activity.All the leaves extract showed significant activity against all pathogens, but the methanolic extract of Tubiflora acaulis Kuntze showed maximum zone of inhibition and minimum inhibitory concentration against all the microorganisms due to the presence of various secondary metabolites. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.*
***Keyword:****Tubiflora acaulis Kuntze,In Vitro Antibacterial, antifungal activities.*

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## INTRODUCTION

Infectious diseases are the major cause of morbidity and mortality and thus a serious public health problem in developing countries. Despite the arsenal of antibiotics available, the situation is worsening due to emerging drug resistance. Antimicrobial resistance in a hospital setting is a major issue these days due to the extensive use or misuse of these drugs [1]. Because of available antimicrobials failure to treat infectious diseases, many researchers have focused on the investigation of natural products as source of new bioactive molecules. A variety of methods are found for this purpose and since not all of them are based on same principles, results obtained will also be profoundly influenced not only by the method selected, but also by the microorganisms used to carry out the test, and by the degree of solubility of each test-compound [2]. India is endowed with a rich wealth of medicinal plants which have been a valuable source of natural products for maintaining human health. A large number of these medicinal plants are used in several formulations for the treatment of various diseases caused by microbes. According to World Health Organization, medicinal plants would be the source of obtaining a variety of drugs. Various societies across the world have shown great interest in curing diseases using plants/ plant based drugs. Microbes are closely associated with the health and welfare of human beings. Some are beneficial and some are detrimental. As preventive and curative measures, plants and their products are used in the treatment of infections for many centuries ago. WHO estimated that 80% of the people worldwide rely on plant based medicines for their primary healthcare and India happens to be the largest user of traditional medical cure, using 7000 plant species [3]. Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not, only because

many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance [4].Thus, in the light of the evidence of the rapid global spread of resistant clinical isolates, the need to find new antimicrobial agents is of paramount importance. However, the past record of rapid, widespread emergence of resistance to newly introduced antimicrobial agents indicates that even new families of antimicrobial agents will have a short life expectancy. Many plants have been used because of their antimicrobial traits, which are due to phytochemicals synthesized in the secondary metabolism of the plant. Plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids, phenolic compounds, and flavonoids, which have been found in vitro to have antimicrobial properties [5].Since ancient times, natural products have been used for the treatment of several infectious diseases. These products including medicinal plant extract are promising sources for the development of novel therapies against diseases [6]. In an effort to expand the spectrum of antibacterial agents from natural resources, *Tubiflora acaulis* Kuntze belonging to Acanthaceae family has been selected. In the Indian literature as per the traditional this plant has been described to be useful by Decoction of root is mixed in equal amount of local liquor and one cup of this mixture is taken daily for 3-4 days in the morning for easy expulsion of guinea worm. Half teaspoon root extract is given to children once a day for two days for asthma by tribal's of Southern Rajasthan [7].*Tubiflora acaulis* Kuntze support folkloric use in the treatment of some. Thus, *Tubiflora acaulis* Kuntze is well anchored in its traditional uses has now found wide-spread acceptance across the world. In the current investigation carried out, a screening of different extracts of *Tubiflora acaulis* Kuntze leaves against pathogenic bacteria and fungi is done in order to detect new sources of antimicrobial agents [8-9]. The present study aimed to evaluate the In Vitro Antibacterial and antifungal activities from leaf extracts of Tubiflora acaulis Kuntze (Acanthaceae) by gram-positive and gram-negative bacteria.

## MATERIAL AND METHODS

### Collection of Plant Materials

The Leaves of *Tubiflora acaulis* Kuntze was collected from Satpuda hills, Akkalkuwa, Dist: Nandurbar, Maharashtra, India, cleaned and dried at room temperature in shade and away from direct sunlight. The dried aerial part was coarsely powdered in grinder. Large difference in particle size of crude drug results in long extraction time as the coarse particles increases the extraction time and fine may form bed, so the powdered material was sieved through 60-120 mesh to remove fine and the powder was subjected for further study.

### Authentication of the plant material

The plant authenticated by Dr. Priyanka A. Ingale, Scientist B, Botanical Survey of India, Pune (Voucher Specimen number-01) by comparing morphological features and a sample voucher specimen of plant was deposited for future reference.

**Fig. 1- *Tubiflora acaulis* KuntzePlant**

### Taxonomy [8]

Kingdom : Plantae
Phylum : Tracheophyta
Class : Magnoliopsida
Order : Scrophulariales
Family : Acanthaceae
Genus : *Tubiflora*
Species : *Tubiflora acaulis*
Scientific name: *Tubiflora acaulis*

### Common names [9]

Marathi : Vismuli, Burandya
Hindi : Patharchatta
Tamil : Pumikatampam
Telugu : Yeddadugu

**Synonym-**

*Elytraria acaulis*

**Preparation of Plant Extract**

The extraction of the Cassia fistula leaves was carried out using known standard procedures. The leaves of *Tubiflora acaulis* Kuntze (Acanthaceae) was collected and dried in the shade and then pulverized in a grinder. Material was passed through 120 meshes to remove fine powders and coarse powder was used for extraction. The powdered was utilized for successive extraction by prescribed in standard reference using Petroleum ether, chloroform and methanol as solvent for extraction of powdered plant[10].The extracts were filtered using Whatman filter paper (No.1) while hot, concentrated in vacuum under reduced pressure using rotary flask evaporator, and dried in a desiccators. The extracts were then kept in sterile bottles, under refrigerated conditions, until further use. The dry weight of the plant extracts was obtained by the solvent evaporation and used to determine concentration in mg/ml.

**Preliminary Phytochemical Screening**

**Qualitative phytochemical analysis**

Phytochemical tests were performed using Petroleum ether, chloroform and methanol extracts to determine the presence of different phytochemicals following established standard protocol. The plant extracts were subjected for the test of alkaloid-like substances, carbohydrates, fixed oils and fats, glycosides (Cardiac, Anthraquinone, Saponin), phenolic compounds and tannins, proteins and amino acids, flavonoids, lignin, terpenoids, and diterpenes. Qualitative phytochemical examinations were carried out for all extracts of leaves as per the standard methods [10-12].

**Test Microorganisms and Growth Media**

The antibacterial and antifungal activity were asses by taking *Staphylococcus aureus*, *Streptococcus pyogenes , Escherichia coli, Pseudomonas aeruginosa* and *fungal strains Aspergillusniger, Aspergillusclavatus , Candida albicans* in Clinitech Solution Excellence in Clinical Research Lab, Hyderabad were chosen based on their clinical and pharmacological importance. The bacterial and fungal stock cultures were incubated for 24 hours at 37°C on nutrient agar. The bacterial strains were grown in Mueller-Hinton agar (MHA) plates at 37°C (the bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C), whereas the yeasts and molds were grown in Sabouraud dextrose agar and PDA media, respectively, at 28°C. The stock cultures were maintained at 4°C.

**Antimicrobial Activity**

**Determination of zone of inhibition method**

In vitro antibacterial and antifungal activities were examined for Petroleum ether, chloroform and methanol extracts of leaves of *Tubiflora acaulis* Kuntze (Acanthaceae). Antibacterial and antifungal activities of extracts against four pathogenic bacteria (two Gram-positive and negative) and three pathogenic fungi were investigated by the agar disk diffusion method. The antimicrobial activities of the crude extracts were determined by agar well diffusion method. Immediately after autoclaving, the media was allowed to cool at 45 to 50°C. The freshly prepared and cooled media was poured into flat-bottomed Petri dishes (90 mm in diameter) and placed on a level and horizontal surface to give a uniform depth of almost 4 mm. The agar media was allowed to cool and solidify at room temperature and the plates were incubated at 35°C for 18–20 h before they were used to confirm sterility. Then 0.1 mL of the tested inoculum was evenly spread on the surface of the solidified agar by using sterile spreader. Four equidistant wells of 8 mm in diameter and 3 mm in depth were made on the agar plate. All the extracts were screened for their antibacterial and antifungal activities against the *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pyogenes* and the fungi *Candida albicans, Aspergillus niger,* and *Aspergillus clavatus*. The sets of five dilutions (5, 25, 50, 100, and 250 µg/ml) of *Tubifloraacaulis* Kuntze extract and standard drugs were prepared in doubledistilled water using nutrient agar tubes. About 100 µL of the Petroleum ether, chloroform and methanol extracts of leaves of *Tubiflora acaulis* Kuntze (Acanthaceae) was filled into the wells. Control experiments were carried out under similar condition by using ampicillin, chloramphenicol, ciprofloxacin, and norfloxacin for antibacterial activity and griseofulvin and nystatin for antifungal activity as standard drugs. The bacterial agar plates were incubated aerobically for 24 h at37 °C. The fungal *C. albicans,A. niger, A. clavatus* plates were incubated for 48 h at 30 °C. Theantimicrobial activities were determined by measuring the diameters of inhibition zones (mm). The sensitivities of the microorganism species to the plant extracts were determined by

measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms [4, 13, 14].

## RESULTS AND DISCUSSION

### Preliminary Phytochemical Screening

### Qualitative phytochemical analysis

Qualitative phytochemical analyses were carried out on *Tubiflora acaulis* Kuntze leaf separately for different extract like pet. Ether, chloroform and methanol and it was found that Petroleum ether extract contain steroids,alkaloids the chloroform extract contain Saponin glycosides, alkaloids, the methanolic extract contain saponins, alkaloids, glycosides, flavonoids, tannins, carbohydrates, proteins and amino acids, steroids.

### Antimicrobial activity

In the present study, the antimicrobial activity of extracts of*Tubiflora acaulis* Kuntze were studied in different concentrations (5, 25, 50, 100, and 250 µ g/ml) against four pathogenic bacterial strains, two Gram-positive (*Staphylococcus aureus, Streptococcus pyogenes*) and two Gram-negative (*Escherichia coli, Pseudomonas aeruginosa*), and three fungal strains (*Aspergillusniger, Aspergillusclavatus, Candida albicans*). These strains have been selected for the basis of its application purpose of further study. Antibacterial and antifungal potential of extracts were assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial and antifungal activities are presented in Tables 1 to 4.

The extracts of *Tubiflora acaulis* Kuntze were increased linearly antibacterial and antifungal activities of with increase in concentration of extracts (µg/ml). As compared with standard drugs, the results revealed that the methanolic extract shows the good growth inhibition zone as bacterial activity of against the strain of *E. coli, P. aeruginosa, S. pyogenes* and *S. aureus* were found to be 26 ± 0.3, 26 ± 0.5, 25 ± 0.3 and 25 ± 0.3 respectively at 250 µg/ml and antifungal activity of *A. niger, A. clavatus* and *C. albicans* were found to be 27 ± 0.8, 27 ± 0.6 and 26 ± 0.3 respectively. The results show that the methanolic extracts of *Tubiflora acaulis* Kuntze were found to be more effective against all the microbes tested.

**Table.1: Antibacterial activity of Different Extract by Agar well diffusion Method**

| Sample with code | Antibacterial activity | | | | |
|---|---|---|---|---|---|
| | Zone of inhibition in mm | | | | |
| | Concentration in µg/ml | *E. coli* | *P. aeruginosa* | *S. pyogenes* | *S. aureus* |
| Pet. Ether Extract (PP) | 5 µg/ml | NA | NA | NA | NA |
| | 25 µg/ml | NA | NA | NA | NA |
| | 50 µg/ml | 8 ± 0.5 | 10 ± 0.6 | 9 ± 0.3 | 10 ± 0.3 |
| | 100 µg/ml | 10 ± 0.8 | 11 ± 0.5 | 9 ± 0.5 | 10 ± 0.5 |
| | 250 µg/ml | 12 ± 0.3 | 13 ± 0.8 | 12 ± 0.6 | 11 ± 0.3 |
| Chloroform Extract (PC) | 5 µg/ml | NA | NA | NA | NA |
| | 25 µg/ml | 10 ± 0.3 | 11 ± 0.3 | 10 ± 0.6 | 12 ± 0.3 |
| | 50 µg/ml | 14 ± 0.5 | 14 ± 0.5 | 14 ± 0.8 | 15 ± 0.6 |
| | 100 µg/ml | 18 ± 0.3 | 15 ± 0.6 | 17 ± 0.3 | 17 ± 0.5 |
| | 250 µg/ml | 20 ± 0.8 | 19 ± 0.3 | 20 ± 0.5 | 19 ± 0.3 |
| Methanol Extract (PM) | 5 µg/ml | NA | NA | NA | NA |
| | 25 µg/ml | 13 ± 0.5 | 12 ± 0.8 | 12 ± 0.5 | 13 ± 0.3 |
| | 50 µg/ml | 15 ± 0.8 | 17 ± 0.5 | 15 ± 0.8 | 13 ± 0.6 |
| | 100 µg/ml | 22 ± 0.6 | 20 ± 0.8 | 20 ± 0.5 | 22 ± 0.6 |
| | 250 µg/ml | 26 ± 0.3 | 26 ± 0.5 | 25 ± 0.3 | 25 ± 0.3 |

Values are means of three replicates. NA: No activity exhibited by extracts against microorganism.

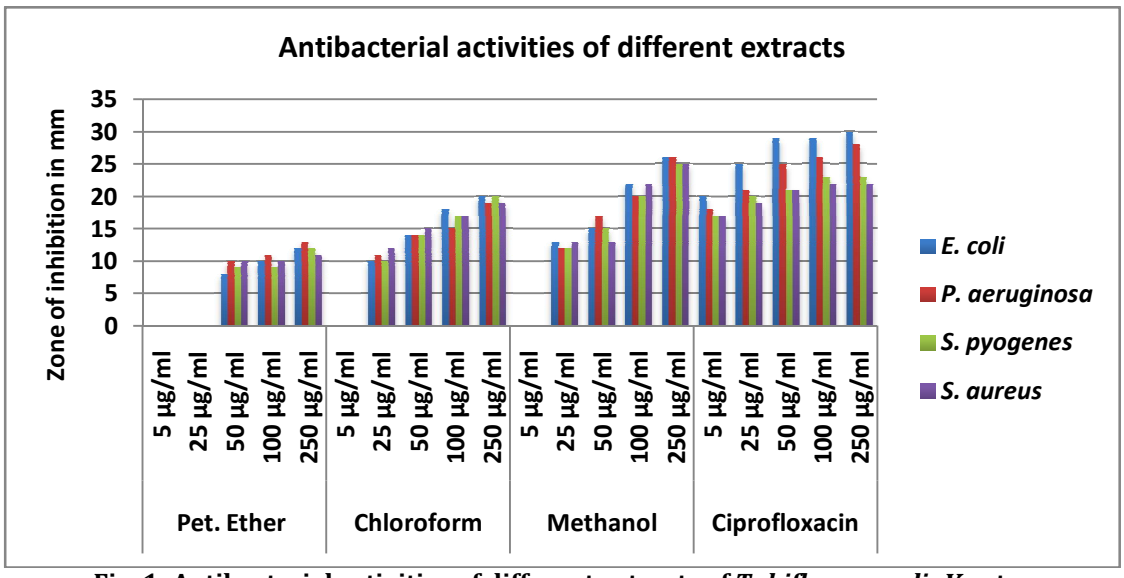


**Fig. 1: Antibacterial activities of different extracts of *Tubiflora acaulis* Kuntze**

**Table 2: Antibacterial activity of Standard drug by Agar well diffusion Method**

| Standard Drug | Antibacterial activity (Zone of inhibition) | | | | |
|---|---|---|---|---|---|
| | Standard Drug Zone of inhibition in mm | | | | |
| | Concentration in µg/ml | *E. coli* | *P. aeruginosa* | *S. pyogenes* | *S. aureus* |
| Ampicillin | 5 µg/ml | 15 ± 0.5 | 15 ± 0.8 | 14 ± 1.2 | 14 ± 0.5 |
| | 25 µg/ml | 16 ± 0.5 | 16 ± 0.8 | 17 ± 1.0 | 16 ± 0.5 |
| | 50 µg/ml | 18 ± 0.3 | 18 ± 0.3 | 17 ± 0.3 | 18 ± 0.3 |
| | 100 µg/ml | 21 ± 0.3 | 21 ± 1.15 | 20 ± 0.5 | 21 ± 0.8 |
| | 250 µg/ml | 22 ± 0.3 | 23 ± 0.5 | 23 ± 0.3 | 22 ± 0.3 |
| Chloramphenicol | 5 µg/ml | 20 ± 0.5 | 21 ± 0.3 | 21 ± 0.3 | 20 ± 0.5 |
| | 25 µg/ml | 19 ± 1.15 | 20 ± 1.5 | 22 ± 0.8 | 19 ± 1.1 |
| | 50 µg/ml | 23 ± 0.8 | 23 ± 0.3 | 24 ± 0.5 | 23 ± 0.8 |
| | 100 µg/ml | 25 ± 1.0 | 26 ± 0.8 | 26 ± 1.0 | 25 ± 1.0 |
| | 250 µg/ml | 29 ± 0.5 | 30 ± 0.3 | 30 ± 0.8 | 29 ± 0.5 |
| Ciprofloxacin | 5 µg/ml | 20 ± 0.8 | 20 ± 1.0 | 19 ± 0.8 | 20 ± 0.8 |
| | 25 µg/ml | 24 ± 0.8 | 24 ± 0.3 | 23 ± 0.6 | 22 ± 0.6 |
| | 50 µg/ml | 26 ± 0.5 | 26 ± 0.3 | 26 ± 0.3 | 26 ± 0.5 |
| | 100 µg/ml | 28 ± 0.5 | 29 ± 0.8 | 28 ± 1.5 | 28 ± 0.5 |
| | 250 µg/ml | 30 ± 0.5 | 31 ± 0.8 | 31 ± 0.8 | 31 ± 0.3 |
| Norfloxacin | 5 µg/ml | 20 ± 0.8 | 19 ± 1.0 | 19 ± 1.0 | 20 ± 0.8 |
| | 25 µg/ml | 23 ± 1.2 | 23 ± 0.8 | 22 ± 0.3 | 23 ± 1.2 |
| | 50 µg/ml | 25 ± 0.6 | 26 ± 0.8 | 25 ± 1.2 | 25 ± 0.6 |
| | 100 µg/ml | 27 ± 0.8 | 28 ± 0.5 | 28 ± 0.5 | 27 ± 0.8 |
| | 250 µg/ml | 31 ± 1.2 | 32 ± 0.8 | 31 ± 0.5 | 31 ± 1.2 |

Values are means of three replicates. NA: No activity exhibited by extracts against microorganism.

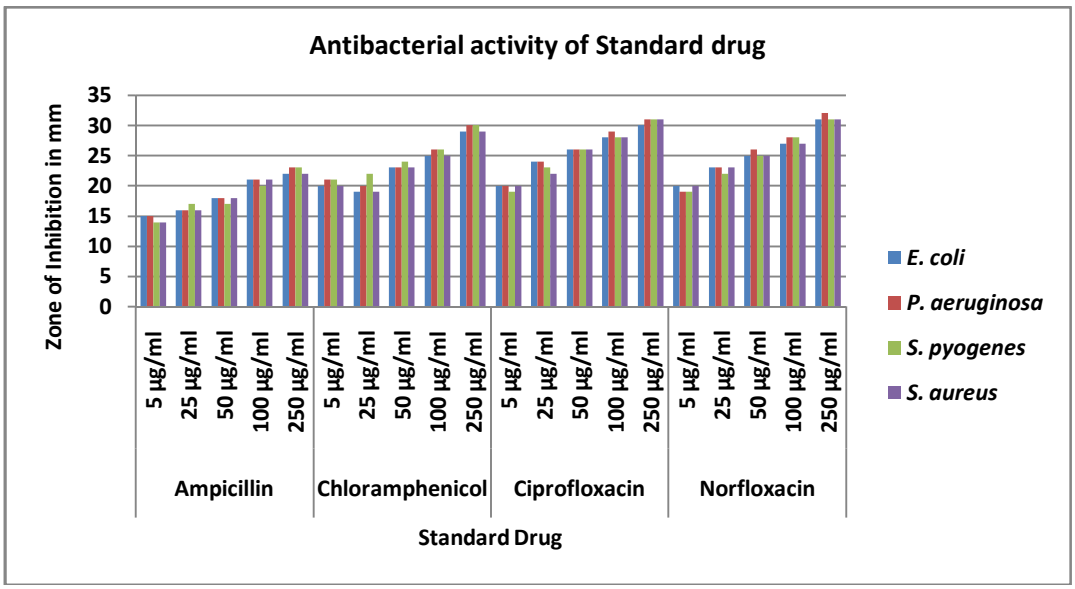


**Fig. 2: Antibacterial activities of Standard drug**

**Table 3: Antifungal activity of Different Extract by Agar well diffusion Method**

| Sample with Code | Antifungal activity | | | |
|---|---|---|---|---|
| | Zone of inhibition in mm | | | |
| | Concentration in µg/ml | *A. niger* | *A. clavatus* | *C. albicans* |
| Pet. Ether Extract (PP) | 5 µg/ml | NA | NA | NA |
| | 25 µg/ml | 9 ± 0.3 | 9 ± 0.3 | 9 ± 0.3 |
| | 50 µg/ml | 10 ± 0.8 | 11 ± 0.6 | 10 ± 0.6 |
| | 100 µg/ml | 14 ± 0.3 | 14 ± 0.5 | 15 ± 0.5 |
| | 250 µg/ml | 16 ± 0.3 | 16 ± 0.3 | 15 ± 0.3 |
| Chloroform Extract (PC) | 5 µg/ml | NA | NA | NA |
| | 25 µg/ml | 10 ± 0.5 | 9 ± 0.5 | 9 ± 0.3 |
| | 50 µg/ml | 14 ± 0.5 | 15 ± 0.8 | 15 ± 0.8 |
| | 100 µg/ml | 21 ± 0.8 | 20 ± 0.3 | 21 ± 0.5 |
| | 250 µg/ml | 22 ± 0.3 | 23 ± 0.5 | 22 ± 0.3 |
| Methanol Extract (PM) | 5 µg/ml | NA | NA | NA |
| | 25 µg/ml | 10 ± 0.5 | 10 ± 0.5 | 10 ± 0.6 |
| | 50 µg/ml | 16 ± 0.3 | 17 ± 0.3 | 17 ± 0.5 |
| | 100 µg/ml | 22 ± 0.6 | 21 ± 0.6 | 22 ± 0.8 |
| | 250 µg/ml | 27 ± 0.8 | 27 ± 0.6 | 26 ± 0.3 |

Values are means of three replicates. NA: No activity exhibited by extracts against microorganism.

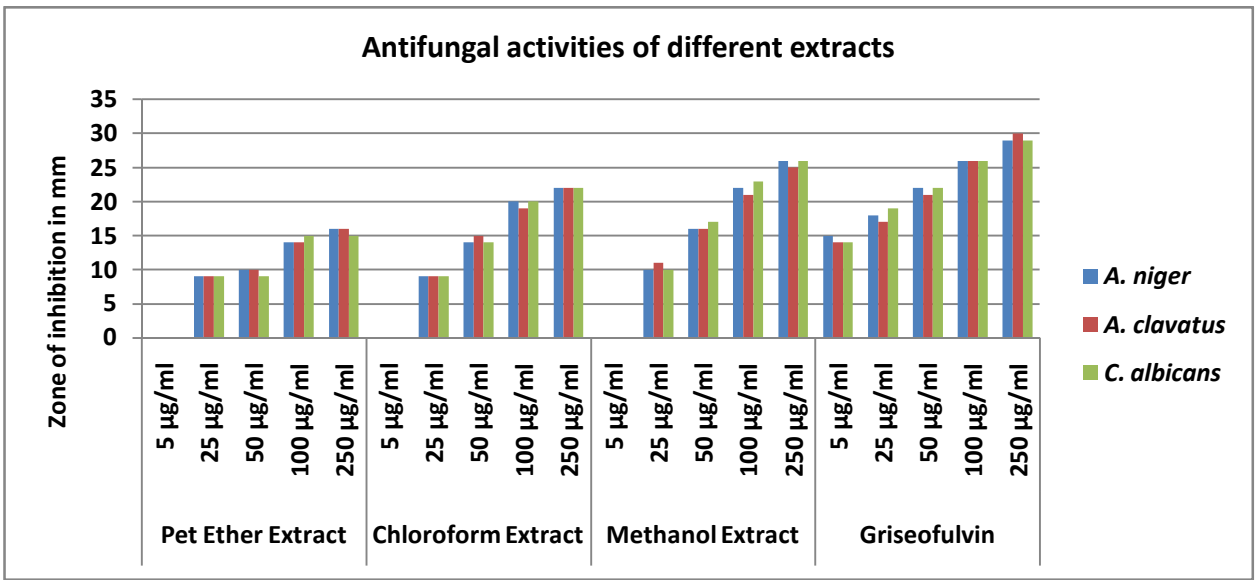


**Fig. 3: Antifungal activities of different extracts of *Tubiflora acaulis* Kuntze**

**Table 4: Antifungal activity of Standard drug by Agar well diffusion Method**

| Standard Drug | Antifungal activity | | | |
|---|---|---|---|---|
| | Standard Drug Zone of inhibition in mm | | | |
| | Concentration in µg/ml | *A. niger* | *A. clavatus* | *C. albicans* |
| Griseofulvin | 5 µg/ml | 15 ± 0.5 | 14 ± 0.8 | 12 ± 0.8 |
| | 25 µg/ml | 19 ± 0.5 | 17 ± 1.1 | 20 ± 0.3 |
| | 50 µg/ml | 21 ± 1.2 | 22 ± 1.2 | 23 ± 0.5 |
| | 100 µg/ml | 26 ± 0.8 | 26 ± 0.3 | 26 ± 0.8 |
| | 250 µg/ml | 29 ± 0.5 | 31 ± 1.0 | 30 ± 0.5 |
| Nystatin | 5 µg/ml | 16 ± 1.4 | 13 ± 0.6 | 16 ± 0.8 |
| | 25 µg/ml | 19 ± 0.5 | 17 ± 0.6 | 20 ± 0.5 |
| | 50 µg/ml | 22 ± 0.8 | 22 ± 0.5 | 24 ± 0.8 |
| | 100 µg/ml | 27 ± 0.8 | 27 ± 0.8 | 28 ± 0.5 |
| | 250 µg/ml | 31 ± 1.1 | 31 ± 0.8 | 31 ± 0.8 |

Values are means of three replicates. NA: No activity exhibited by extracts against microorganism.

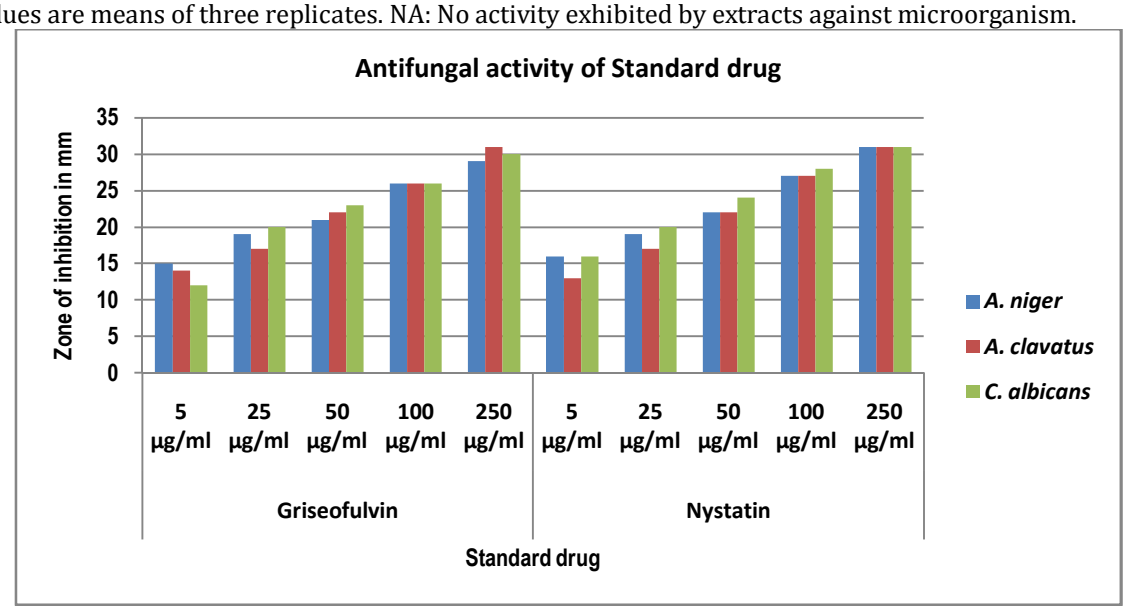


**Fig. 4: Antifungal activities of standard drug**

## DISCUSSION

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism [15].

In the present investigation, different extracts of *Tubiflora acaulis* Kuntze were evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive bacteria, fungus which was regarded as human pathogenic microorganism. Susceptibility of each plant extract was tested by agar well diffusion method.

Our preliminary investigation showed that all Petrolium ether, chloroform and methanol extracts of *Tubiflora acaulis* Kuntze were active against the locally isolated human pathogens like *Escherichia coli, Staphylococcus aureus, Bacillus cereus*, and *Pseudomonas aeruginosa* but among them methanolic extract shows the maximum growth inhibition zone when compared to standard drug. Similarly as antifungal activity against*A. niger, A. clavatus* and *C. albicans* were shows the maximum growth inhibition zone in methanolic extract.

Thus the methanolic extracts of *Tubiflora acaulis* Kuntze showed significant antimicrobial activity against multi-drug resistant clinically isolated microorganisms (Fig. 1-4)

This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the present investigation showed that the plant contains more or less same components like saponin, triterpenoids, steroids, glycosides, anthraquinone, flavonoids, proteins, and amino acids. Results show that plant rich in tannin and phenolic compounds have been shown to posses' antimicrobial activities against a number of microorganisms.

## CONCLUSION

In the present work, in vitro studies concluded that extracts i.e. petroleum ether, chloroform and methanol extracts inhibited fungal growth and growth of gram negative and gram positive bacteria. This result may provide a basis for the isolation of compounds from this plant. Further studies are needed to identify the purecomponent and establish the mechanism of action for antimicrobial activity of different parts of the plant with different extracts.The present study justified theclaimed uses of leaves in the traditional system of medicineto treat various infectious disease caused by the microbes.

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