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# INVESTIGATION ON PHYTOCHEMICAL AND ANTIMICROBIAL PROPERTIES OF ACORUS CALAMUS EXTRACT

*R.M. Balaji* [1*] *and D. Shanthakumari*[2]
Department of Biochemistry, Research & Development Centre, Bharathiar University, Coimbatore - 641 046.
Dept. of Biochemistry, Indira Gandhi Jayanthi Women's College, Kilgudalore, Tindivanam-604 307.
[*]*Corresponding author*: balagbio@gmail.com

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## Abstract

Acorus calamus is a prominent perennial flora often employed in the traditional healthcare system and have long been familiar for the therapeutic value. It's a semi-aquatic and smelly flora which is exist in both the temperate as well as sub-temperate zones. Antimicrobic action of *Acorus calamus* rhizome samples attained using various solvents viz., $H_2O$, Methanol, Ethanol, Butanol, Hexane, etc. were assessed. The phyto-chemical study exposed the existence of Flavonoids, Glycosides, Saponins, Resins and Steroids in the rhizome extract and it was assumed to confer antimicrobial activities. The Extracts attained with Ethyl Acetate and Ethanol amongst others were identified as hugely efficient. Ethanol and Ethyl acetate extracts showed pronounced antimicrobial activity towards MRSA by a significant zone of inhibition and antifungal action towards *Aspergillus niger* by a significant zone of inhibition. The rhizome extracts can be employed as potential antimicrobial agents.

## Introduction:

The ordinary products have been widely employed for treating of various infectious human disorders for about a millennium, meanwhile they reveal a wide ranging of biological assets, which can be utilized in the medical care system. In the timeline of evolution, the Microorganisms have developed resistance to many antimicrobial agents giving rise to the battle of humans versus the drug resistant infections thereby creating an enormous medical

problematic in the therapy strategy for infectious disorders. This resist has significantly enlarged owing to the excessive utilization of commercially available antimicrobic drugs. The therapeutic plants signify an ironic reservoir of antimicrobic mediators and these are utilized in traditional medicine systems in different countries and can serve as alternatives for potent and powerful drugs.

The scientific interest in plants for therapeutically and pharmacologically significant active elements is on the rise at a rapid rate. The therapeutic floras are planting whose 2º metabolites are potential resources of curative medicines. The flora delivers an excess of several natural substances, like the flavonoids, alkaloids, or the isoprenoids that can often be connected with the bioactive assets of the floras. This plant derived organic products were examined widely and they are known to play a major part in numerous useful bio-activities, like the antimicrobial, antifungal, antiyeast, pesticides and herbicides and they was well recognized. Several ongoing researches on the examination of bio-activities of plant-based products. These biologically active mixtures serve as the lead compounds in the growth of several inorganic potential antibiotics [1].

The WHO estimated that around 80 percent of the worldwide population utilizes the natural medicine system for their 1º medical care. India is the 8$^{th}$ principal country that is richer in terms of floral diversity by a nearly 47,000 organisms that greater than 7500 organisms were extensively employed as therapeutic floras. The floral substances were the major resource of drug around the worldwide for treatment of numerous human ailments. Around 50% of the modern drugs in the USA are resultant from various plants [2]. The usage of traditional drug in both the emerging as well as established countries is pointedly and there was a rising request for drugs of Ayurveda, Siddha, Unani and Homeopathy for national and exporting determinations.

*Acorus calamus* is a very prominent flora in the Indian traditional medicines [3] for periods. Both inorganic and plant-based substances extracted from a number of sources have been detected and produced as possible drugs over the last 2 centuries. One such significant herb reported to have antibacterial activities is Acorus calamus, which belonging to the Acoraceae family. A number of names, like cinnamon sedge, flag root, myrtle grass, gladdon, myrtle, etc. are descriptively recognized. It is a medicinal grass via an aromatic rhizome, cylindrical, rising to 2.5 cm thick, purple- to light-brown outside and whiter inside, spreading and widely branched. The A. leaves. Calamus has a model is deemed mid vein on both ends and several fine tertiary veins, and mildly higher secondary veins. This distinctly separates it from Acorus americanus. Betting on the leaf among 0.7 and 1.7 cm in width. The flora is very hardly exhibit flower or set fruit and the flowers were 3 to 8 cm long, cylindrical in size, green cum brown and coated in an assembly of rounded spikes. The fruits were tiny and berry-based, comprising a very tiny seeds [4].

The A. calamus possibly endemic to the Indian sub - continent, is also present in Europe, Southern Russia, China, Japan, Minor Northern Asia, and so on. The Calamus is an appetite drug, bitter herb and helps metabolism. The decoction is being used in North America for fevers, stomach cramps and colic; toothaches and dried rhizomes are chomped on the rhizome is usually breathe in for congestion. In the Ayurvedic system the *Calamus* is a significant herb, and it was esteemed as a "rejuvenator" for the mind and nervous system. The parts that are generally used medicine system are the leaves, root (rhizome) and stem. The rhizomes have been used in Ayurvedic therapeutic practice in India to treat a variety of ailments, such as fever, asthma and bronchitis, but also as a sedative [5]. The herb is usually expressed in Western herbal remedies for digestive issues like gas, bloating, colic, and poor digestive function [4]. With this background information the purpose of the current work was to evaluate the Phyto-chemical and Antibacterial behaviour of rhizome sample of *Acorus calamus*.

## Material and Methods:

### Collection of plant material:

*A. calamus* Linn (Fig.1) is commonly known as the sweet flag belongs to the *Araceae* family. The pure rhizomes of the plant *Acorus calamus* (Fig.2) were attained from Bharathiar University, Coimbatore, Tamil Nadu India.

**Fig. 1*: A. calamus***

**Fig.2: Rhizomes of *A. calamus***

**Bacteria Cultures:**

The microbes used in our work was medical isolates of the infective microorganisms. The cultures include Methicillin Resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* (ATCC 259220), *Pseudomonas aeruginosa* (ATCC 27853), Clinical isolates - *Salmonella paratyphi* and *Klebsiella*, two fungal strains - *Candida albicans* ATCC7596 and *Aspergillus niger* ATCC9763. All the tubes inoculated with these organisms were incubated at room temperature for 48 hours.

Microbe was cultured on Mueller Hinton agar, MHA (Himedia, Mumbai) at 35ºC. Fungal was cultivated and sustained on Sabouraud's dextrose agar (SDA) (Himedia, Mumbai) at 25 and 35 ºC.

**Preparation of plant powder:**

The Rhizome portion of the floral substance was washed in running tap water. Tiny hairs of *A. calamus* were detached and the rhizomes were sliced, dry at lower temperatures and then normalized to finer powdered and stockpiled in airtight flasks.

**Extract preparation:**

An existing quantity of the rhizome powdered (4 g) was collected discretely with diverse diluents viz., Aqueous, Methanol, Ethanol, Butanol, Hexane, Petroleum ether and Ethyl acetate for 24 hrs according to the procedures that were issued in earlier studies [6]. The Extracts thus prepared were weighed and then subjected to storage at 4ºC.

**Phytochemical Study:**

Total extracts procured were considered for the initial phyto-chemical study by employing the standard protocol for the identification of diverse phyto-constituents [7]. The Phytochemical tests were done for the determination of the class of compounds that are present in the active fractions and can be assumed to be responsible for the characteristic activities of the plant. Accordingly, the presence of Flavonoid, Glycoside, Saponin, and so on were determined by performing the standard protocols. The results were reported as (+) for presence, and (−) for absence.

**Overall Flavonoid Constituent**

The overall flavonoids exist in the solvent was evaluated through the Aluminium chloride method published by Brighente et al., by minor modifications [8]. 1 ml of the rhizome solvent was mix with equal capacity of 2 percent Aluminium chloride and then was subjected to incubation at room T for about 15 mins. The absorbance of this mix was evaluated at 430nm in UV spectrophotometry towards the blank. Quercetin was utilized for the calibration

of the standard curve. The tests were performed in triplicates and the outcomes were stated as mg/g Quercetin equivalent.

**Total Glycosides content:**

Tiny quantity (0.5g) of the solution was dispersed in 2 ml of chloroform and conc. $H_2SO_4$ was prudently introduced to create the down layer. A reddish-brown colour at the interphase shows the existence of a steroidal ring of the glycoside.

**Total saponins content:**

5 ml of distilled $H_2O$ was introduced to about 0.5 g of the extract in a testing tube. The solution was then agitated vigorously and was measured for a constant determined froth. The frothing was then mix with about 3 droplets of olive oil and was agitated vigorously after that it was detected for the creation of an emulsion. A (+) sign was documented when the froth touched a height of about 0.5 cm; a (++) symbol for a height of about 0.6 - 1 cm; and a (+++) mark for a height of greater than 1cm, showing less, moderate or higher conc. of saponins, respectively, in the floral extract.

**Total Resin content:**

1 ml of various solvent extracts were salted with few droplets of acetic anhydride solution surveyed by 1 ml of concentration $H_2SO_4$. Resins provide colouration that ranges from orange to colour of yellow.

**Total Steroids content:**

(Salkowski test) 2ml of the extract and 1ml of concentrated H2SO4 acid was introduced carefully along with the ends of the test tubes. The formation of red-colour shows the occurrence of steroids.

**Antibacterial activity:**

The disk diffusion method [9] was employed for screening the antibacterial activity. Sterile 6-mm diameter paper disks were permeated by 10-mg (10 µl) of the extract thawed in 95 percent ethanol. Air dry plates were kept in an injected MHA surface. Commercially accessible antibiotic impregnated disks of Vancomycin (30 µg) and Gentamicin (10 µg) were utilized as standard antibiotics and disks infused by 10 µl of 95 percent ethanol as -ve controls. Plates are subjected to incubation at 35º C for 18 hrs and the suppression zone width was restrained. The experiments were accomplished in duplicates. The Minimum Inhibitory Concentrations or MICs were achieved through the improved agar-based dilution technique [9]. Different concentration of (100mg per 500µl, 50mg per 500µl, 25mg per 500µl, 12.5mg per 500µl and 6.25mg per 500µl) rhizome sample were prepared in 1 ml working solution of extract was added. In order to achieve a total concentration of approximately 100-0.78 mg/ml in 9 cm width disks, serial 2-fold titrations of β-asarone were combined

by molten MHA in the 1:100 ratio. The agar layer was mounted on the sterile filter membrane. The suspension of the inoculum (2 µl) was injected by a multipoint inoculator (104 CFU/spot) on the filter membrane. Cells were placed for 18 h at 35ºC. By reading the smallest concentration that hindered visible development, the MICs were captured. The membrane-based filter was further placed into a fresh MHA cells were seeded for 18 h at 35ºC. By interpreting the smallest concentration that hindered visible development, the MICs were captured. The membrane filter also was placed onto a fresh MHA cells were seeded for 18 hrs at 35ºC. Minimum bactericidal concentrations (MBCs) were reported as the least conc. on the β-asarone fraction-free sheets that did not show development.

**Antifungal study:**

The antifungal study towards yeasts were accomplished similar to the protocol of the antimicrobial assays discussed above by the alternative of SDA as an assay media and then minimum fungicidal concentrations (MFCs) were measured. Amphotericin B (Biochem pharmaceutical industries Ltd., Mumbai) was employed as a +ve control. Disks were nurtured at 35 ºC for 24 hrs (*C. albicans*) and 48 hrs (*A. niger*). The Hyphal development suppression study was utilized for the determination of the antifungal behaviour. The methodology employed in the hyphal development suppression test has been already published in an earlier study [10]. Briefly, the dilutions of testing solutions dispersed in 95 percent ethanol was introduced to the sterile molten SDA at 45 ºC at a proportion of 1:100 to provide last concentrations of 1, 0.8, 0.6, 0.5, 0.4, 0.2 and 0.1 mg per ml. The resulting solvent is carefully combined and then about 100 µl was released onto each sterilized 1.5-cm width known microscope slides. Plugs of 1 mm of the fungi mycelium cut from end of actively developing growth was injected at the centre part of the agar-based well and nurtured in a moist compartment at 25 ºC. The control microbes attained an equal amount of 95 percent ethanol. 8 duplicates were employed for every conc. Radial development was evaluated once the control species nearly touched the end of wells and these outcomes was stated as percentage of hyphal development suppressed [11]. The conc. response curves were processed in which the % of the hyphal growth suppression was designed towards the concentration.

**Results:**

The results exhibited the presence of phytoconstituents such as Flavonoid, Glycoside, Saponin, Resin and Steroid as shown in the table 1. Numerous researches recommended the occurrence of 2º metabolite to have exposed few bioactivities in humans [12]. Flavonoid was present in Methanol, Ethanol, Hexane, Butanol, Petroleum ether and Ethyl acetate solvents and absent in Aqueous. Saponins were found to be present in the Aqueous, Methanol, Ethanol, Hexane, Butanol and Ethyl acetate extracts and absent in Petroleum ether. The resin and glycosides were highly present in Ethanol and Butanol. Steroid was present in overall extract and highest was present in

Methanol, Ethanol and Ethyl acetate. By doing the phytochemical analysis the present study clearly suggested that the bioactive constituents can be accountable for the antibacterial action of the rhizome solvents.

**Table 1: Phytochemical analysis of rhizome extract of *A. calamus***

| Phytochemicals | Plant Extracts | | | | | | |
|---|---|---|---|---|---|---|---|
| | Aqueous | Methanol | Ethanol | Hexane | Butanol | Petroleum ether | Ethyl acetate |
| Flavonoid | - | + | + | + | + | + | + |
| Glycoside | + | + | + | - | + | + | + |
| Saponins | + | + | + | + | + | - | + |
| Resin | + | - | + | + | + | + | + |
| Steroid | + | + | + | + | + | + | + |

**Antibacterial activity of rhizome extracts:**

The antibacterial activity of *A. calamus* was found high in Ethyl Acetate and Ethanol extracts. Ethyl Acetate and Ethanol demonstrated greatest zone of inhibition towards *Pseudomonas aeruginosa* and MRSA. Antimicrobial action towards *Salmonella paratyphi* and E. coli was exhibited higher in the Petroleum-based ether and Ethyl acetate. Minimum antibacterial activity of rhizome extract was found against *Klebsiella*.

**MICs of rhizome extracts:**

After the remark of antimicrobial action of floral extract, we further analysed the MIC of rhizome solvent of different solvents. For these we processed the extract at diverse conc. by serial-based dilution technique. The outcomes of MIC towards diverse microbe were listed in the table 3. From this table 2 it was strong in which the rhizome solvent of *A. calamus* shows higher antibacterial activity against MRSA and *E. coli* than the *S. paratyphi, P. aeruginosa* and *Klebsiella*. In the case of Methanol and Aqueous solvents of rhizome, *A. calamus* showed minimum antimicrobial action towards *Klebsiella, P. aeruginosa, S. paratyphi, E. coli* and MRSA. Petroleum ether solvent depicts medium antimicrobial action towards *E. coli and* lesser towards against *S. paratyphi, Pseudomonas, MRSA* and *Klebsiella.* MIC was measured for 100mg/500µl. Ethanol and Ethyl acetate extracts showed highest zone of inhibition against MRSA (17-21mm). Despite of the fact that MRSA is a resistant bacterium, the Ethyl acetate extract of rhizome showed significant (21mm) zone of inhibition. The overall studies of antimicrobial action showed

the ethyl acetate and ethanol are moderately efficient towards the MRSA, *E. coli*, and *S. paratyphi.*

**Table 2: Zone of Inhibition of *MRSA, E.coli, Pseudomonas aeruginosa, Salmonella paratyphi* and *Klebsiella* against rhizome extracts of *A. calamus* in (mm).**

| Plant Extracts | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Aqueous** | **Methanol** | **Ethanol** | **Hexane** | **Butanol** | **Petroleum ether** | **Ethyl acetate** | **Vancomycin** | **Gentamicin** |
| 8mm | 11mm | 17mm | 12mm | 10mm | 12mm | 21mm | 29mm | 33mm |
| 9mm | 10mm | 14mm | 6mm | 8mm | 14mm | 16mm | 28mm | 30mm |
| 8mm | 9mm | 10mm | 9mm | 8mm | 9mm | 11mm | 26mm | 27mm |
| 9mm | 11mm | 12mm | 10mm | 12mm | 10mm | 13mm | 26mm | 28mm |
| 6mm | 8mm | 7mm | 8mm | 8mm | 9mm | 12mm | 25mm | 26mm |

**Table 3: MIC of rhizome extracts against MRSA.**

| **Extracts** | **Minimum inhibitory concentration** | | | | |
|---|---|---|---|---|---|
| | **6.25mg/500µl** | **12.5mg/500µl** | **25mg/500 µl** | **50mg/500µl** | **100mg/500µl** |
| Aqueous | 5mm | 7mm | 9mm | 10mm | 13mm |
| Methanol | 8mm | 6mm | 10mm | 12mm | 14mm |
| Ethanol | 9mm | 10mm | 12mm | 15mm | 17mm |
| Hexane | 8mm | 9mm | 11mm | 13mm | 15mm |
| Butanol | 9mm | 10mm | 11mm | 14mm | 16mm |
| Petroleum ether | 5mm | 6mm | 10mm | 11mm | 12mm |
| Ethyl acetate | 12mm | 15mm | 17mm | 19mm | 21mm |

## Anti-fungal activity of rhizome extracts:

The anti-fungal behaviour of the rhizome solvent of *A. calamus* were depicted high in ethanol, butanol and Ethyl acetate solvents towards *A. niger* but less towards *C. albicans*. In that, no antifungal behaviour of $H_2O$ and Petroleum ether solvents towards *A. niger* and *C. Albicans* (Table.4).

**Table 4: Zone of Suppression of *Aspergillus niger* and *Candida albicans* toward rhizome solvents of Acorus calamus.**

| Culture | Plant Extracts | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Aqueous | Methanol | Ethanol | Hexane | Butanol | Petroleum ether | Ethyl acetate | Amphotericin B |
| *A. niger* | 8mm | 14mm | 20mm | 16mm | 19mm | 11mm | 22mm | 25mm |
| *C. albicans* | 6mm | 9mm | 12mm | 6mm | 14mm | 6mm | 7mm | 22mm |

## MIC of rhizome solvents towards fungus:

We tested the MIC of rhizome extracts from various solvents after noticing the antifungal behaviour of crude extracts. For this purpose, we processed the extract at various concentration in the form of serial-based dilution technique. The outcomes of the MIC towards the fungi *A. niger* were shown in table 6. Rhizome solvent of *A. calamus* is hugely effective towards *A. niger* than *C. albicans*. Ethyl acetate extract of *A. calamus* demonstrated higher antifungal action towards *A. niger* than *C. albicans*. Butanol and ethanol extracts are quite effective against both *A. niger* and *C. albicans*. Petroleum ether, methanol and water solvents did not show any antifungal action towards *A. niger* and *C. albicans*.

**Table 5: MIC of rhizome sample towards *A. niger*.**

| Solvents | Minimum inhibiting concentration | | | | |
|---|---|---|---|---|---|
| | 6.25mg/5 00µl | 12.5mg/50 0µl | 25mg/500 µl | 50mg/500µ l | 100mg/500µl |
| Methanol | 6mm | 7mm | 10mm | 12mm | 14mm |
| Ethanol | 8mm | 8mm | 12mm | 15mm | 20mm |
| Hexane | 8mm | 10mm | 12mm | 13mm | 16mm |
| Butanol | 7mm | 8mm | 9mm | 14mm | 19mm |
| Ethyl acetate | 8mm | 9mm | 12mm | 16mm | 22mm |

## Discussion:

An alarming increase has been found in the number of microbial species emerging resistance to a wide range of antibacterial agents. This difficulty that a rehabilitated strategy be completed to seek antimicrobial agents efficient towards these infective microbial resistance to the present antibiotics. That most of these plants were examined theoretically for the antibacterial

activities and a significant number of bioactive compounds have been documented to suppress bacterial growth of the bacterial infections. A significant number of such agents exhibit mechanisms and mechanism of action which are distinctive from that of the antibiotics in the actual usage, indicating that cross tolerance with both the agents already accessible may be limited [13]. Several previous as well as current work have designated numerous significant bioactivities, specifically the antibacterial efficacy of *A. calamus* roots, Rhizome and essential oils [14-15]. In this study *A. calamus* was used for the antimicrobial action towards MRSA, *E. coli, P. aeruginosa, S. paratyphi, Klebsiella* and also antifungal action towards *A. niger* and *C. albicans*. Although our present study only further strengthened and supported the previously published reports on the useful biological properties of the *A. calamus,* some reports have recommended that the substances like Flavonoid, Glycoside, and so on are present in the natural sources. The overall analysis of the results obtained here clearly indicated that the rhizome predominantly possessed bioactivities (Antibacterial and Anti-fungal) when compared to any other plant parts such as leaf that has less bioactive effects (Table 2). Different extracts of *A. calamus* were used for this study. Fascinating outcomes were identified in the circumstance of *A. calamus*. MIC was measured per 100mg/500µl. Ethanol and Ethyl acetate samples displayed highest zone of inhibition against MRSA 17-21mm respectively. The MIC of Ethanol and Ethyl acetate samples depicted highest zone of inhibition against *E. coli* 14-16mm followed by *S. paratyphi* were 12-13mm and *P. aeruginosa* were 10-11mm respectively. The lowest MIC was found in *Klebsiella* which ranged 7-12mm (Table. 2). Our result supported the finding of Rani A.S et al. [16]. The zone of inhibition identified towards *E. coli* had significant range similar to our study. Previously, De M et al. (1999) [17] on the antibacterial actions of *A. calamus* had stated absence of antibacterial activity while Phongpaichit S et al. (2005) [18] observed very lesser antimicrobial activity in this work on antimicrobial properties of *A. calamus* rhizome and these are in contradiction with our study. In our study results the rhizome samples with ethanol and E.A. towards MRSA was found to be in the range of 17-21 mm. Overall in this study the Ethyl Acetate and Ethanol are more effective against the MRSA and followed by *E. coli, S. paratyphi* and *Klebsiella.*

In our study the Rhizome extract of *A. calamus* was found to be highly effective against *A. niger*. The Ethyl acetate showed high antifungal against *A. niger* than *C. albicans* (Table. 4). Ethanol and Butanol extracts showed high effectiveness against *A. niger* than *C. albicans*. Kumar et al. (2010) [19] has also stated the anti-fungal action in Ethanolic extract of *A. calamus* against *A. niger* and *A. flavus*. Our analysis showed zone of inhibition against *A. niger* is in contradiction to the outcomes of Kumar et al. (2010) in which zero activity was observed. Overall, in our study the antifungal action of rhizome sample of *A. calamus* in variety of solvent were analysed and it was found that Ethyl acetate and Ethanol extracts displayed a superior antifungal activity. Depends on our outcomes, we can achieve that ethanol and ethyl acetate sample of *A. calamus* rhizome has high potential antibacterial and antifungal activity.

## Conclusion:

*Acorus calamus* is a prominent perennial flora often employed in the traditional healthcare system and have long been familiar for the therapeutic value. The present study was designed to examine the Phyto-chemical and Antimicrobial action of rhizome extract of *Acorus calamus*. In the Ayurvedic therapeutic preparation India, the rhizomes are utilized to combat numerous disorders namely the fever, asthma and bronchitis, and also as the sedative. From our results it is clearly evident that the *A. calamus* exhibits antibacterial and antifungal activity which can be further exploited for therapeutic purposes in different medicine systems. Methanol and Aqueous extracts of rhizome, *A. calamus* showed minimum antimicrobial action towards *Klebsiella, P. aeruginosa, S. paratyphi, E. coli* and MRSA. Petroleum ether solvent depicts modest antimicrobial action towards *E. Coli and* lesser towards *S. paratyphi, Pseudomonas, MRSA* and *Klebsiella.* In our study the Rhizome extract of *A. calamus* was found to be highly effective against *A. niger*. The overall analysis of the results obtained here clearly indicated that the rhizome predominantly possessed bioactivities (Anti-bacterial and Anti-fungal) when compared to any other plant parts such as leaf that has less bioactive effects. The Ethyl acetate showed high antifungal towards *A. niger* than *C. albicans.* Ethanol and Butanol extracts showed high effectiveness against *A. niger* than *C. albicans* This work tends to express that the active ingredients of the rhizome portion may be good removed with Ethanol and Ethyl acetate extract than other solvents. In view of the richer variety of this floral, it can be anticipated that the screening and technical assessment of the floral extract for their antibacterial action can deliver newer insights for the development of novel antimicrobial compounds. The Medicinal property of *A. calamus* is owing to the incidence of $2^{o}$ metabolites and this can be further studied for its therapeutic potential in alternative to the commercially available antibiotics and antifungal agents.

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