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In vitro Antimicrobial Effect of Essential oil from Leaf and Rhizome of Various Accessions of *Acorus calamus* Linn., and Its Phytochemical Screening

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AK designed the study, wrote the manuscript, data analysis and interpretation. Authors SP and JC contributed in execution, data collection and the literature searches. Author RK was supervisor of the study, Data analysis and overall responsibility. All authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to analysis phytochemical constituents and antimicrobial effect of the essential oil of the leaf and rhizome obtained from the various accessions of *Acorus calamus* L., collected from India. Studies were carried out in C. G. Bhakta Institute of Biotechnology, Uka Tarsadia University, India, between June 2013 and August 2014.

Different plant accessions of *A. calamus* were collected from India. The extraction was carried out by hydrodistillation in a Clevenger-type apparatus. Chemical test for the screening of bioactive chemical constituent in plant was carried out using acetone-water extract. The essential oils were scanned in the wavelength ranging from 200-800 nm by using Ultra Visible spectrophotometer and Fourier transform infrared spectroscopy (FTIR). The disk diffusion method and minimal inhibitory concentrations (MICs) were used to screen the antimicrobial effect of essential oils various accessions.

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The phytochemical screening revealed the various group in plant such as steroids, alkaloids, tannins, phenols, flavanoids, fatty acid, cardiac glycosides, carbohydrate, amino acid and proteins. Anthocyanin and leucoanthocyanin were present only in leaf essential oils. UV spectrum data recorded maximum absorbance peak ranges between 205 nm to 363 nm. FTIR spectrum showed the peaks in the range of 587.72- 2996.41 cm^{-1}. Essential oils from rhizome and leaf were screened for its antimicrobial activity against different microbial strains. Rhizome essential oil was shown to have higher activity than leaf oil. Except *Enterobacter aerogenes* all the test microorganisms were significantly inhibited by almost all the essential oils even at very low MIC. All different accessions from different states showed inhibition but the plants obtained from North India (PWB, HW, ROH, DAN) showed the maximum Percentage of Inhibition.
The analysis carried out on this plant shows that the plant could be explored as a potential antimicrobial drug in phytomedicine.

Keywords: Antimicrobial activity; Acorus calamus; UV-VIS and FTIR spectrum analysis; Non-metric multidimensional scaling (NMDS).

1. INTRODUCTION

Our interest in plants because of their medicinally and pharmacologically important active ingredients is increasing rapidly. The plant produces a plethora of natural products, such as alkaloids, phenolics, flavonoids and terpenoids or isoprenoids which has often been correlated with medicinal and pharmacological properties of the plants. These plant natural products have been studied extensively and their role in several useful biological activities such as antibacterial, antifungal, antiyeast, insecticidal and herbicides has been well documented [1]. There are 7500 species being used as medicinal plants in India [2]. From those, *Acorus calamus* L., (Family: Araceae) is a perennial plant with creeping and extensively branched, aromatic rhizome, cylindrical, up to 2.5 cm thick, purplish-brown to light brown externally and white internally. The leaf of *A. calamus* has a single prominent midvein and then on both sides slightly raised secondary veins and many, fine tertiary veins. Plants very rarely flower or set fruit, but when they do, the flowers are 3 to 7 cm long, cylindrical in shape, greenish brown and covered in a multitude of rounded spikes. The fruits are small and berrylike, containing few seeds [3]. The common names of *A. calamus,* which are used in different parts in India, are Bach (Hindi), Vasambu (Tamil), Baje (Kannada), and Vasa (Telugu). The plant is found in the northern temperate and subtropical regions of Asia, North America, and Europe. The plant prefers swampy or marshy habitats. It is plentiful in the marshy tracts of Kashmir, Himachal Pradesh, Manipur, and Naga hills, and is regularly cultivated in Karnataka. The plant exhibits polyploidy and three karyotypes (diploid, triploid, tetraploid). In India, the plant is found growing wild as well as cultivated up to an altitude of 2200 m in the Himalayas [2]. The tetraploid plant oil is high in β- asarone (90-96%). The triploid plants contain a small portion of β-asarone (5%) in their oil and the diploid plants lack β-asarone [4]. Studies of chemical composition of *Acorus* spp. have revealed that α- and β-asarones are the major active components. Various bioactivities are attributed to β-asarone, like antibacterial, anthelmintic, and antifungal properties [5]. *A. calamus* leaf, rhizomes and its essential oil has many biological activities [2] like antispasmodic, carminative and also used for treatment of epilepsy, mental ailments, chronic diarrhea, dysentery, bronchial catarrh, intermittent and tumors. It also has the insecticidal, antifungal, antibacterial [6] tranquilizing, antidiarrhoeal, antidyslipidemic, neuroprotective, antioxidant, anticholinesterase, spasmolytic, vascular modulator activities [7]. With this knowledge, the present project work was aimed to produce the UV-VIS and FTIR spectrum profile with antibacterial and antifungal activity of essential oil of leaf and rhizome of various accessions of *A. calamus*.

2. MATERIALS AND METHODS

2.1 Collections of Plant Materials

Different plant accessions of *A. calamus* were collected manually from different states of India such as Meghalaya, Uttarakhand, Himachal Pradesh, Maharashtra, Jammu, Punjab and Karnataka and were maintained in Green house in Maliba campus is located at 21°.04 N 73°.03 E. (Table 1) Bardoli has an average elevation of 22 meters (72 feet) and at an altitude of 34.18 MSL above mean sea level. The voucher specimens were deposited at the herbarium of the same department. The fresh leaves and rhizomes were collected, washed with running

tap water followed by sterilized distilled water and shade dried and powdered using mortar and pestle.

2.2 Essential Oil Extractions from Whole Leaf and Whole Rhizome

The extraction was carried out by measuring 20 g of *A. calamus* leaf and rhizome powder respectively in 200 ml of distilled water and the essential oil was obtained by hydro distillation for three hours in a Clevenger-type apparatus [8], until no more condensing oil could be seen. The essential oil ranging in rhizomes from 3.6% - 5.5% (v/w) and 1.7% - 2.9% (v/w) in leaf were separated from the aqueous solution (hydrolate), and the essential oil was stored at 4°C for further analysis(Table 1).

Percentage of oil content = (Weight of oil extract/ Weight of sample) × 100 [9]

2.3 Tests for Qualitative Determination of Bioactive Compounds of *A. calamus*

Chemical test for the screening and identification of bioactive chemical constituent in the *A. calamus* plant under study were carried out using acetone and water extract.

2.3.1 Acetone-water extract

500 g of coarse powder of leaf and rhizomes of *A. calamus* were mixed with 300 ml of distilled water and 300 ml of acetone in cold for 2 days. The solvent from the total extract was filtered and the filtrate was concentrated on a water bath for 3 hours, remaining filtrate was used for phytochemical analysis [10]. The individual extract was subjected to the qualitative phytochemical screening for the presence of some chemical constituents. Phytochemical test was carried out a adopting standards procedure [10,11].

2.3.1.1 Steroid

1 ml extract was dissolved in 10ml of chloroform and equal volume of concentrated H_2SO_4 acid was added from the side of test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence this indicates the presence of steroid.

2.3.1.2 Tannin

2 ml extract was added to 1% lead acetate a yellowish precipitate indicates the presence of tannins.

2.3.1.3 Saponin

5 ml extract was mixed with 20 ml of distilled water then agitated in graduated cylinder for 15 min formation of foam indicates saponin).

2.3.1.4 Anthocyanin

2 ml of aqueous extract is added to 2 ml of 2 N HCl & NH_3, the appearance of pink red turns blue violet indicates presence of anthocyanin.

2.3.1.5 Coumarin

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates coumarins.

2.3.1.6 Alkaloids

A quantity (3 ml) of concentrated extract was taken into a test tube and 1 ml HCl was added then the mixture was heated gently for 20 min cooled and filter, the filtrate was used for following test.

2.3.1.7 Hager's test

Filtrate was treated with Hager's reagent, presence of alkaloids confirmed by the yellow colored precipitate.

2.3.1.8 Proteins

Xanthoproteic test: extract was treated with few drops of concentrated HNO_3 formation of yellow indicates the presence of proteins.

2.3.1.9 Amino acids

Ninhydrin test: to the 2 ml extract 2 ml on ninhydrin reagent was added & boil for few minutes, formation of blue colour indicates the presence of amino acid.

2.3.1.10 Phytosterol

Salkowski's test: extract was treated with chloroform and filtered. The filtrate was treated with few drops of concentrated H_2SO_4 and shakes, allow standing, appearance of golden red indicates the positive test.

2.3.1.11 Phenol

Ferric chloride test: test extract were treated with 4 drops of alcoholic $FeCl_3$ solution. Formation of bluish black color indicates the presence of phenol.

2.3.1.12 Phlobatannins

Deposition of red precipitate when aqueous extract of each plant sample is boiled with 1% aqueous HCl was taken as evidence for presence of phlobatannins.

2.3.1.13 Leucoanthocyanin

5 ml of Isoamyl alcohol added to 5 ml of aqueous extract, upper layer appear red in colour indicates the presence of leuanthocyanin.

2.3.1.14 Cardial glycosides

Keller-Killani test: plant extract treated with 2 ml Glacial acetic acid containing a drop of $FeCl_3$. A brown colour ring indicates the positive test.

2.3.1.15 Flavonoid

Alkaline reagent test: extract was treated with 10% NaOH solution, formation of intense yellow color indicates presence of flavonoid.

2.3.1.16 Fatty acids

The extract was mixed with 5ml of ether. These extract was allowed for evaporation on filter paper and filter paper was dried. The appearance of transparency on filter paper indicates the presence of fatty acids.

2.4 Spectroscopic Analysis

To detect the active component using UV-VIS spectrum of the essential oil of different accessions of *A. calamus*, the essential oils were scanned in the wavelength ranging from 200-800 nm by using a Ultra-visible spectrophotometer and the characteristic peaks were detected. The extracted volatile essential oil of the leaves and rhizomes was subjected to the FTIR analysis using a Bruker spectrophotometer, (USA) system which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FTIR were recorded. Each and every analysis was repeated thrice for the spectrum confirmation.

2.5 Microbial Stains

The pure culture of experimental bacteria was obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India. Test organisms used were *Escherichia coli* (MTCC 40), *Staphylococcus aureus* (MTCC 3160), *Bacillus subtilis* (MTCC 1305), *Enterobacter aerogenes* (MTCC 2823) and *Bacillus megaterium* (MTCC 428). Fungal mycellium *Penicillium chrysogenum* (MTCC 161), *Rhizopus oryzae* (MTCC 4615), *Trichoderma koningii* (MTCC 8611), *Aspergillus niger* (MTCC 281) and *Aspergillus flavus* (MTCC 277) were used for antifungal activity. The pure cultures of bacteria were maintained on nutrient agar medium and fungi on Rose Bengal Agar medium. Each culture was maintained by sub culturing regularly on respected medium and stored at 4°C, before use in experiments.

2.6 Antibacterial Assay

The disk diffusion method was used to screen the antibacterial and antifungal activity of essential oils various accessions. Petri dishes (100 mm) containing 20 ml of Mueller- Hinton agar (Hi media, India) seeded with 0.2 ml inoculums of bacterial stains, (inoculums size was adjusted so as to deliver a final inoculums of approximately 10^7cfu mL^{-1}.Media was allowed to solidify and then individual Petri dishes were marked for the bacteria inoculated. Sterile 6-mm diameter paper disks were impregnated with 10µl of essential oil of leaf and rhizome of different accessions dissolved in hexane (1:1) then disks were placed on the inoculated MHA surface. Hexane and sterilized distilled water were used as negative control while commercially available antibiotic disks of Streptomycin (30µg/ml) were used as positive control. Plates were incubated at 37°C for 24 h and the inhibition zone diameter was measured.

2.7 Antifungal Assay

Sterile 6-mm diameter paper disks were impregnated with 10 µl of essential oil of leaf and rhizome of different accessions dissolved in Hexane (1:1) Air-dried disks were placed on the inoculated Rose Bengal agar plate (Hi media,India). Hexane and sterilized distilled water were used as negative control while commercially available Amphotericin B (50 µg/ml) was used as positive control. Each fungal mycellium was cut (1mm) from the edge of the active growing colony and were inoculated in the center of a Rose Bengal agar plate Plates were incubated at room temperature for 5-7 days. Radial growth was measured when the control colonies almost reached the edge of the plate wall [12]. Results were expressed as the percentage of hyphal growth inhibited by using the formula [13].

The antimicrobial tests were performed in triplicate. The zone of inhibition at different accessions of essential oils was carefully studied

in comparison with the zone of inhibition with control petri plates [6].

2.8 Determination of Minimum Inhibitory Concentration

The minimal inhibitory concentrations (MICs) of essential oils of various accessions were determined by disc diffusion method. Essential oils producing an inhibition zone ≥15 mm in diameter were screened to MICs by standard two-fold broth dilution methodology given by NCCLS [14]. The serial dilution of essential oils of all the accessions by dissolving in hexane was carried out in different dilutions were added at a ratio ranges from 0.36 µg/ml to 0.55 µg/ml for rhizome and 0.17 µg/ml to 0.29 µg/ml for leaf oils to 0.036 µg/ml to 0.055 µg/ml for rhizome and 0.017 µg/ml to 0.029 µg/ml for leaf oils respectively [14,15]. MICs for antifungal assay, the hyphal growth inhibition test were used to determine antifungal activity. The resultant solution was thoroughly mixed and approximately 10µl was dropped into each sterile paper disk (6mm diameter) and placed on agar plate [12]. Results were expressed as the percentage of growth inhibited [13]. Inoculations and incubation conditions were same as mention above.

3. RESULTS AND DISCUSSION

Preliminary results obtained for qualitative screening of phytochemicals of *A. calamus* leaf and rhizomes are presented in Table 2. Out of the fifteen phytochemicals screened twelve were found present in the acetone extract of *A. calamus* rhizome. They are alkaloid, carbohydrates, cardiac glycosides, flavonoids, phenols, phlobatannins, amino acids, proteins, saponins, sterols, tannins and coumarin but anthocyanin and leucoanthocyanin were present only in leaf essential oils [1]. *In vitro* efficacy of bioactive extracts of various medicinal plants were studied previously [16] and acetone fraction extract in most of the cases exhibited higher potency (low MIC value) as compared to ethyl acetate, hexane and methanol fraction [17]. From numerous previous studies it has become clear that the difference in the effectiveness could be due to the solvent used for extraction and isolation of bioactive compound from the concerned medicinal plant parts or climate and geographical difference [18]. This test reveals the presence of phenols and flavonoids groups in the extract which was further confirmed by FTIR and UV-VIS spectroscopy. UV spectrum data for essential oil of *A. calamus* for all the accessions recorded maximum absorbance peak ranges between 205 nm and 363 nm and characteristic absorbance peak at 0.824 and 0.416 respectively indicates highly phenolic nature. Similar type of results were observed in several other research work [1].

In FTIR spectrum profile shows the fingerprint region of the all accessions carried out using essential oils, spectral analysis revealed fingerprint region of all essential oil was almost identical with the fingerprint region of standard Asarone (Sigma-Aldrich, USA) spectrum profile. FTIR spectra of all the accessions showed peaks in the range of 587.72- 2996.41 cm^{-1} for OH group of phenolic compound which may be constitute the structure of asarone present in the samples [19]. However, it is not worthy to mention here that results were obtained in FTIR alone are not significant to prove the existence of compound-classes, especially when it comes to mixture of many compounds.

The essential oils and extracts of *A. calamus* have been found to possess an antibacterial activity [20,21]. The antibacterial activities of the essential oils of various accessions are shown in Fig. 3. All the five organisms tested had given inhibitory zone Fig.1 excluding *Enterobacter* sp., by all the essential oils. However the sensitivity varied among the four organisms, high percent inhibition were observed in *Bacillus subtilis* (56.50% by leaf oil and 98.50% by rhizome oil) followed by *Bacillus megaterium* (12.05%-32.05% by leaf oil and 25.0%-64.0% by rhizome oil), and *Staphylococcus aureus* (8.41%-17.26% by leaf oil and 16.80%-34.52% by rhizome oil). *E. coil* showed low percentage inhibition ranges 7.75%-32.05% by leaf oil and 15.50%-31.84% shown in Fig. 3 and Fig. 4. Although both leaf and rhizome of *A. calamus* showed antibacterial acidity but It had no activity against *Enterobacter aerogenes* [2]. The DHW oil sample produced highest zone (56.50% (L) and 98.50 % (R)) was observed in *Bacillus subtilis* and PWB, HW, ROH and SBL oil samples also produced zone (47.50%(L) and 92.24%-95.00%(R) was observed in *B. subtilis.* Other oil samples SHI, NGP, JAT, JUC, AK, DAN and RRL also produced good inhibitory zone in *B. subtilis.* The both oil samples HW and NGP were produced larger zone against *B. megaterium.* The HW, NGP, DAN, ROH, SBL oil samples also given better activity against *S. aureus* and *E. coli.* In present study MIC of leaf essential oil was ranges 0.36 µg/mL to 0.55 µg/mL for rhizome and 0.17 µg/mL to 0.29 µg/mL for leaf oils for *B. subtilis, B. megaterium, S. aureus* and *E. coli*

shown in Fig. 3 and Fig. 4. In DAN accession essential oils from rhizome was <0.55 µg/mL against *S. aureus* hile it was 0.24 µg/mL of leaf essential oils against *B. subtilis, B. megaterium* (Fig. 2). The antimicrobial activity of *A. calamus* essential oil highlights the importance of natural resources [21]. The antibacterial effect of the rhizome oil of *A. calamus* tested against the in vitro growth of the fish pathogen *A. hydrophila* is also reported [22].

All the four fungus tested had given inhibitory zone by all the essential oils Fig. 7 *Aspergillus flavus* showing highest antifungal activity against accession RRL(73.33% by leaf oil and 83.33% by rhizome oil) (Figs. 5,6), ROH (82.22% by leaf oil and 70% by rhizome oil), are the accessions showing highest antifungal activity at 1:10 concentration against in MIC. *Trichoderma koningiis* showed lower antifungal activity against SHI (58.88% by leaf oil and 47.77% by rhizome oil) antifungal activity against *Penicillium chrsogenum* ranges from in all accessions (5.88% - 65.22% by leaf oil 56.66% - 74.44% by rhizome oil) shown in Fig. 5 and Fig. 6. Essential oil fractions against four strains of fungus were presented for MIC at the concentration of 0.36 µg/ml to 0.55 µg/ml for rhizome and 0.17 µg/ml to 0.29 µg/ml for leaf oils. And comparing various accessions against test organism: BPL, PIT, SBL, NK show highest inhabitation at 1:10 concentration against *Penicillium chrsogenum*, DAN(0.55 µg/µl), RRL(0.54 µg/µl), show highest inhibition at 0.035% concentration against *Rhizopus oryzae,* PWB, show highest inhibition at 0.54µg/µl concentration against *Trichoderma koningii*, JUC are the accessions showing highest antifungal activity at 0.51 µg/µl concentration against *Aspergillus flavus*, From the above antifungal assays result we found out that RRL, JUC and DAN shows a higher percentage of inhibition against the test organism. Additional NMDS analysis on the effects of *A. calamus* essential oil (Leaf and Rhizome) on various fungus and bacteria. (Fig. 8) showed a statistically significant effect ANOSIM p=0.0001 Clear separation were evident in antimicrobial activities occurred in essential oils of leaf and rhizomes of all accessions of *A. calamus* but overall the rhizome oil showed higher antibacterial activity that leaf oil [2]. Earlier reports on assessment of antimicrobial effect of *A. calamus* extract revealed it potential efficiency antimicrobial effect [23]. The previous studies strongly suggested that *A. calamus* rhizomes and leaves must possess active ingredient β- and α-asarones which is considered to be responsible for their antimicrobial activities [23]. Our present study agreed with this findings because both rhizomes and leaf volatile oils showed antimicrobial effects [20].

Table 1. Collection of various accessions of plant *A. calamus* from Indian germplasm

Accessions	Voucher number	Place of collections	Latitude (^{0}N)	Longitude (^{0}E)	Oils (%)	
					Rhizome	Leaf
SHI	CGBIBT/ ACAK031	Shillong, Meghalaya	25.5667^{0}N	91.8833^{0}E	3.6	1.9
PWB	CGBIBT/ ACAK032	Pantanagar, Uttarakhand	28.97^{0}N	79.41^{0}E	5.4	2.3
DAN	CGBIBT/ ACAK033	Dehradun, Uttarakhand	30.3157^{0}N	78.0336^{0}E	5.5	2.4
HW	CGBIBT/ ACAK034	Hardwar, Uttarakhand	29.96^{0}N	78.16^{0}E	4.1	2.9
ROH	CGBIBT/ ACAK035	Rohru (Shimla), Himachal Pradesh	31.202^{0}N	77.75^{0}E	5.3	2.3
NGP	CGBIBT/ ACAK036	Nagpur, Maharashtra	21.15^{0}N	79.09^{0}E	3.9	1.8
RRL	CGBIBT/ ACAK037	Jammu	32.73^{0}N	78.87^{0}E	5.4	2.3
JAT	CGBIBT/ ACAK038	Jatolivillvagham, Uttarakhand	29.85^{0}N	79.77^{0}E	4.2	2.1
JUC	CGBIBT/ ACAK039	Jalandhar, Punjab	31.3256^{0}N	75.5792^{0}E	5.1	2.2
AK	CGBIBT/ ACAK040	Akola, Maharashtra	23.7^{0}N	77.00^{0}E	4.9	1.7
DHW	CGBIBT/ ACAK041	Dharwad, Karnataka	15^{0}21'42"N	75^{0}05'06"E	5.1	2.5
SBL	CGBIBT/ ACAK042	Bangalore, Karnataka	12^{0}58'N	77^{0}34'E	4.2	1.9

Table 2. Phytochemical screening of leaf and rhizome of *A. calamus*

Tests	SHI		PWB		DAN		HW		ROH		NGP		RRL		JAT		JUC		AK		DHW		SBL	
	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R
Alkaloid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardial Glycosides	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Flavanoids	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phlobatannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Amino acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Protein	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Saponins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sterols	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tannins	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coumarin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fatty acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Anthocyanin	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Leucoanthocyanin	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-

Key: "L" indicates Leaf extract, "R" indicates rhizome extract, "+" indicates presence of bioactive compound and "-" indicates absence of bioactive compound

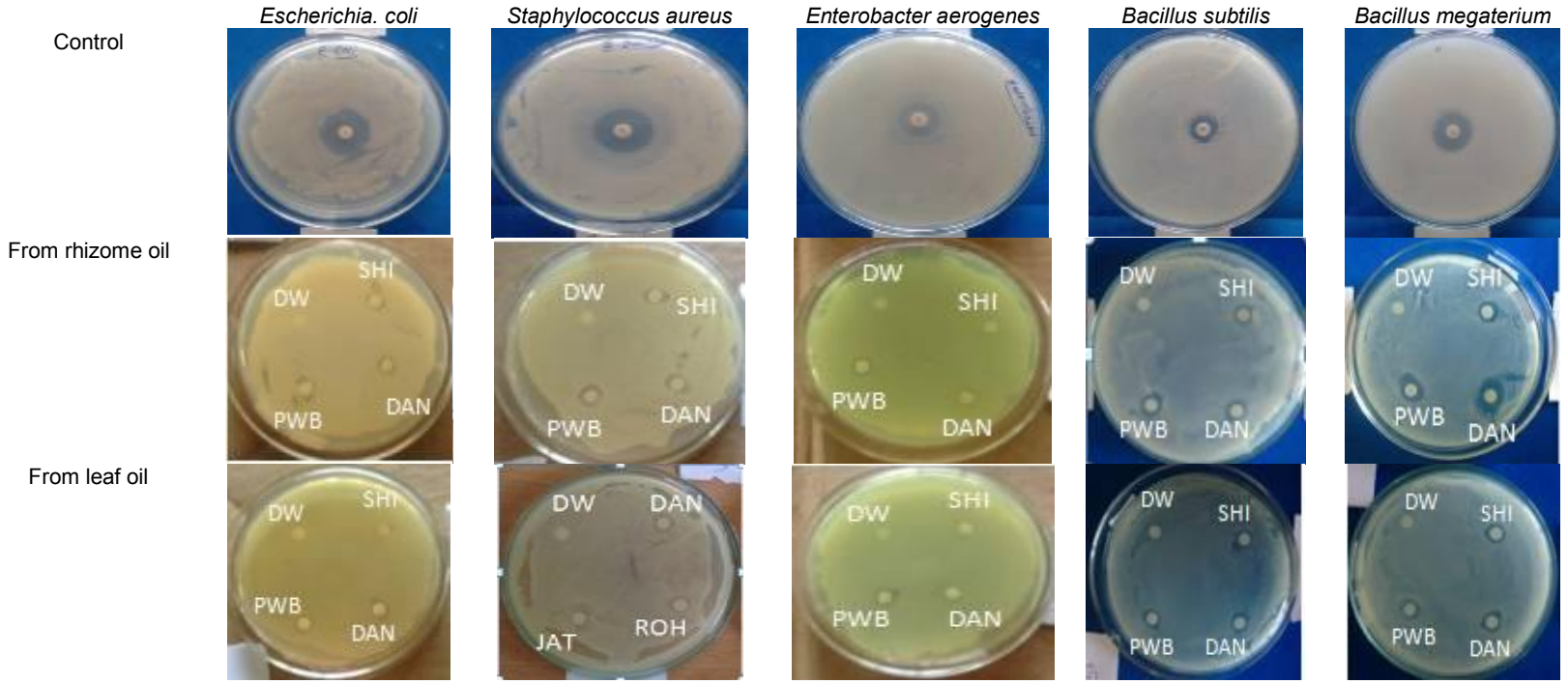
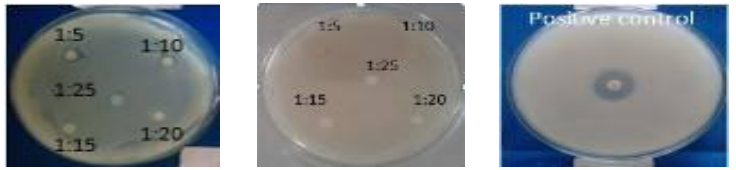


Fig. 1. Antibacterial activity of different accessions of *Acorus calamus* essential oil from leaves and rhizome against various organisms



From rhizome oil From leaf oil

The concentrations taken was
20.0 µg/µl(R); 8.0 µg/µl(L)(1:5),
4.0 µg/µl(R); 1.6 µg/µl(L)(1:10),
0.8 µg/µl(R); 0.32 µg/µl(L)(1:15),
0.16 µg/µl(R); 0.064 µg/µl(L)(1:20)
0.032 µg/µl(R); 0.0128 µg/µl(L) (1:25)

Fig. 2. Minimal Inhibitory Concentration (MIC) at five different concentrations (for SHI sample against S. aureus)
Where, "R" indicates rhizome oil and "L" indicates leaf oil

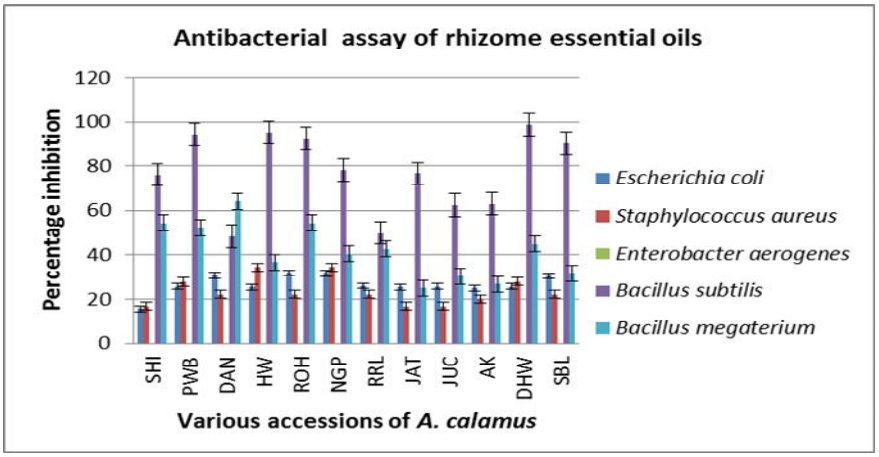


Fig. 3. Comparative analysis of antibacterial activity of different accessions of *Acorus calamus* essential oil from rhizome against various organisms

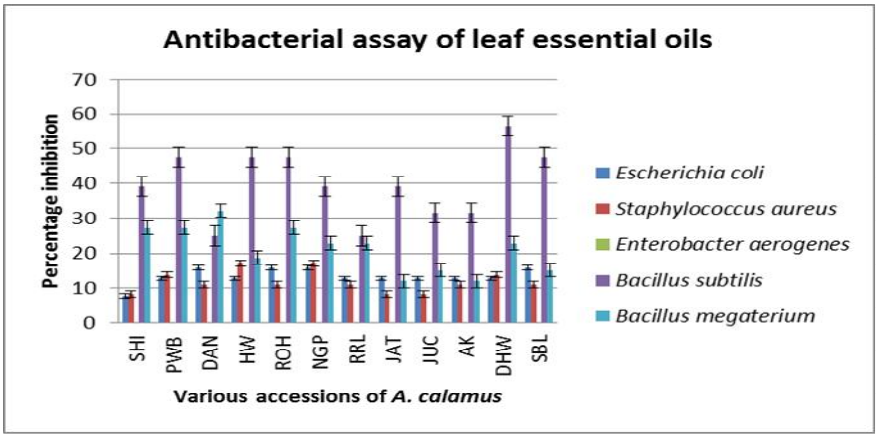


Fig. 4. Comparative analysis of antibacterial activity of different accessions of *Acorus calamus* essential oil from leaves against various organisms

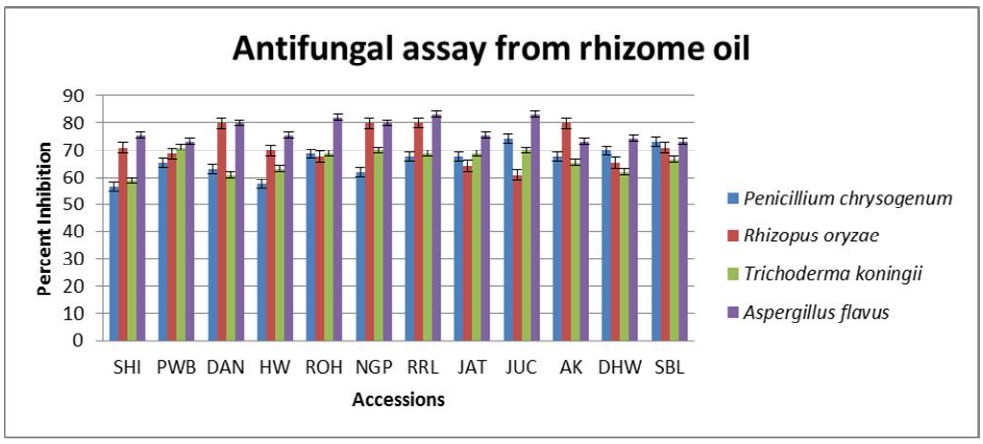


Fig. 5. Comparative analysis of antibacterial activity of different accessions of *Acorus calamus* essential oil from rhizome against various organisms

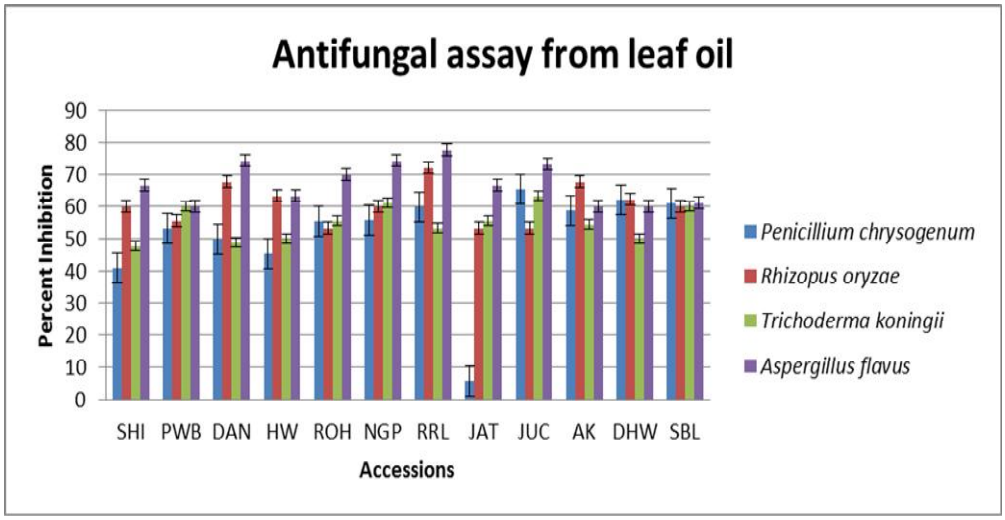


Fig. 6. Comparative analysis of antifungal activity of different accessions of *Acorus calamus* essential oil from leaves against various organisms

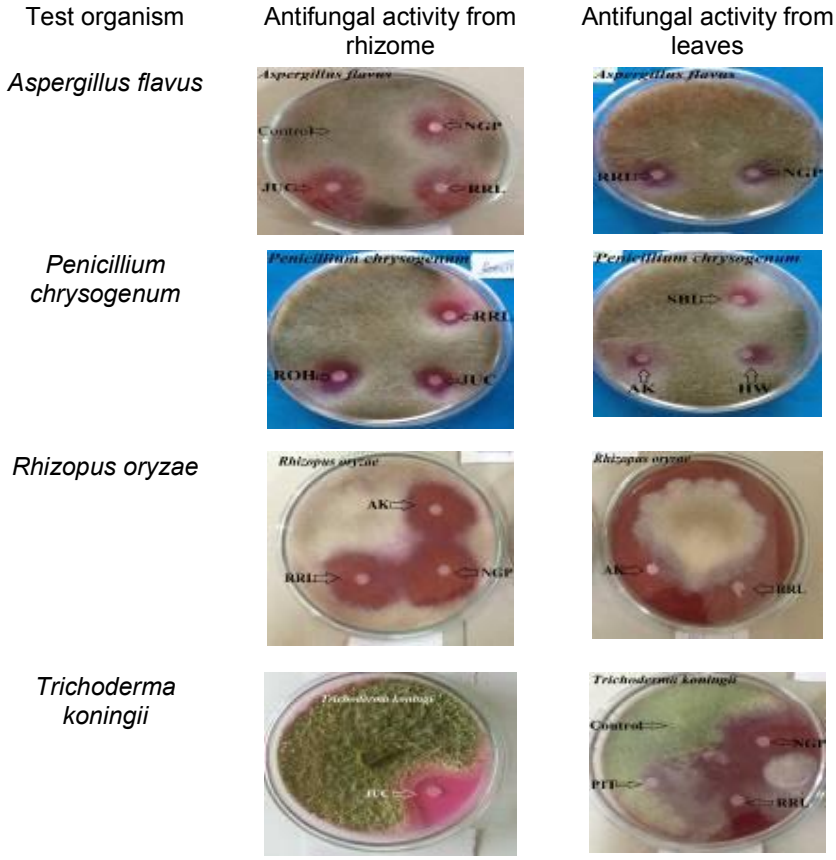


Fig. 7. Antifungal activities of different accessions of *Acorus calamus* essential oil from leaves and rhizome against various organisms

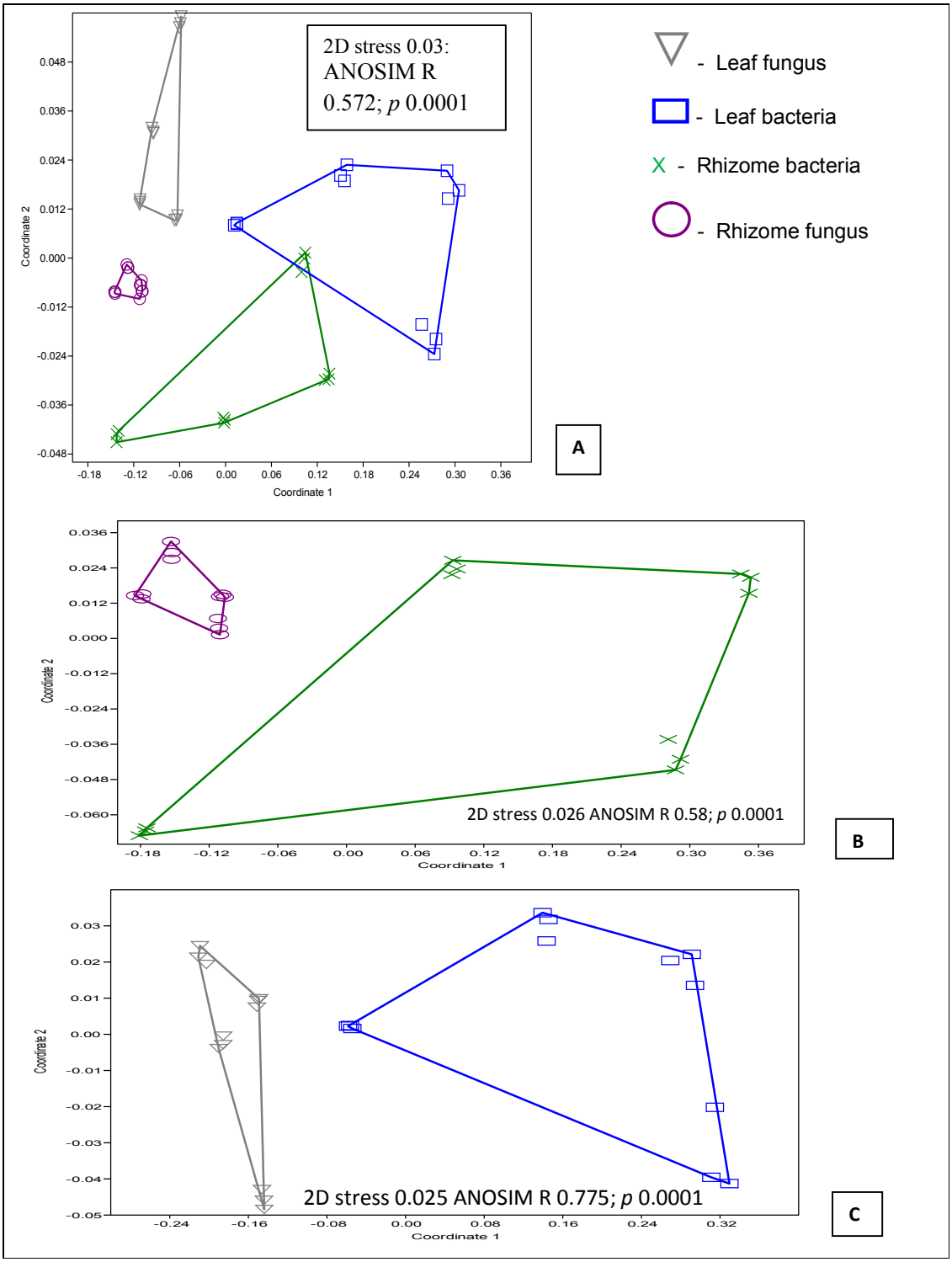


Fig. 8. Non-metric multidimensional scaling (NMDS) analysis of the effects of *A. calamus* essential oil (Leaf and Rhizome) on various fungus and bacteria A - Combine results B - (NMDS) analysis of the effects of *A. calamus* rhizome essential oil on various fungus and bacteria. C - (NMDS) analysis of the effects of *A. calamus* leaf essential oil on various fungus and bacteria

4. CONCLUSION

All antimicrobial effects occurred in essential oils of leaf and rhizomes of all accessions of *A. calamus* was concentration dependent approach as reported by MIC determination, however, and the efficacy of essential oils are lesser than to that of standard antimicrobial, Streptomycin (30 µg/mL) and Amphotericin B (50 µg/mL). This study had shown that rhizome oil give higher activity than leaf oil. The plants obtained from North Indian parts (PWB, HW, ROH, DAN etc.) had given better result than other plants. Results indicated that essential oils of this plant has significant antimicrobial potential against the various bacteria and fungi of clinical significance and authenticates the traditional medicinal value of the *A. calamus*. The essential oils obtained by the *A. calamus* may potentially be used in the treatment of various infectious diseases caused by microorganisms that are demonstrating emergence of resistance to currently available antibiotics. Furthermore, active plant extracts may be subjected to various pharmacological evaluations by several methods such as GC MS (Gas chromatography and Mass Spectrometry) NMR (nuclear magnetic resonance), etc. for the isolation of the therapeutic antimicrobials.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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