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Phytochemical screening and antimicrobial activity of *Erigeron karvinskianus* DC

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Abstract
Erigeron karvinskianus, a small herb, belonging to the family Asteraceae, was identified by the Botanical Survey of India, Shillong, Meghalaya. The plant parts such as stem, roots and leaves were dried, powdered and subjected to Soxhlet extraction with various solvents based on its polarity. Phytochemical screening of the methanolic and aqueous extract detected the presence of alkaloids, flavonoids, phenolic compounds and tannins. The extracts were subject to antimicrobial assay (DPPH) against four micro-organisms, *viz*., *E. Coli*, S. Sureus, A. Niger and C. Albicans. The methanolic extracts of leaves exhibited significant antibacterial activity with zones of inhibition of 19.5mm (500 µg/µl) against *E. coli* (Gentamicin as positive control, 18.5 mm). The aqueous extract of the stem-roots showed effective antifungal activity with zones of inhibition measuring 10.5mm (500 µg/µl) against *A. niger* (Amphotericin B as positive control, 10.0 mm). The study proposes that *E. karvinskianus* can be considered as a potent plant-based antimicrobial agent.

Keywords: *Erigeron karvinskianus*, phytochemical screening, anti-microbial analysis

1. Introduction
Plant kingdoms are rich source of secondary metabolites, many of which have been used for medicinal purposes [1]. For centuries wild plants and animals have played a significant role in satisfying household subsistence needs, including nutrition, medical treatment, and energy supply, among others [2, 3]. These plant resources have been evaluated and maneuvered especially by indigenous tribal communities whose lifestyle is very closely interrelated with nature. This traditional knowledge system is derived from the web of interactions between humans, plants, animals, natural forces, and land forms [2, 4]. India houses 427 such tribal communities, of which more than 130 major tribal communities live in the North-East region [2, 5]. In Northeast India, the areas occupied by the Naga tribal community are considered as part of the Indo-Burma biodiversity hotspot region [6]. The state of Nagaland lies between 25°6´-27°4´N and 93°20´-95°15´E, and has an area of 16,527 sq km (R.R. Rao & N.S. Jamir, 1982). With its complex physiography, Nagaland is endowed with a rich and diverse natural flora and owes this richness to the region in which it falls- A junction of three different geoclimatic zones *viz*., the Indian, the Himalayan and the oriental landmass. The rich floral diversity of the area is largely due to wide altitudinal variation, topographical features, soil characteristics and climatic factors which favored the luxurious growth of plants. The richness of the plant diversity is also evident from the use of varieties of wild edible plant species, fruits and medicinal plants by this hill tribal community [8]. Traditional system of healing practice is highly practiced in the state by using locally available herbs [9]. Many forest areas of the state Nagaland are still yet to be explored scientifically, believed to have a great potential of discovering new plant species [10]. *Erigeron karvinskianus DC* is a species of flowering plant in the family Asteraceae. *Erigeron karvinskianus* is a perennial herb up to 70 centimetres (28 inches) in height, spreading by means of underground rhizomes. It has hairless to hairy leaves reaching up to 10 centimetres long at the base of the branching stem, getting smaller higher up on the stem. The plant usually produces only one flower head per stem, each with 30–80 blue, purple, pink, or white ray florets surrounding numerous disc florets. This plant is found abundantly in Nagaland and can be considered as a weed. However, very little is known about neither the medicinal properties nor its phytochemical constituents.

Phytochemicals are chemical compounds naturally present in the plants attributing to positive or negative health effects [11].The medicinal properties of the plants are determined by the phytochemical constituents [12]. Some of the important phytochemicals include alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes, etc. which are distributed in various parts of the plants [13]. Phytochemicals can be separated from the plant material by various techniques.

Fungal pathogens and bacterial pathogens have become the causes of many pathogenic diseases. They have become major clinical problems. Increased consideration has been focused on the usage of natural anti-microbial agents, especially from plant origins, due to their safety and efficacy as well as the fact that the majority of these plants are classified as generally recognized as safe [1]. Therefore, in the recent times multiple studies have been conducted for extraction of secondary metabolites from plant origins. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [14].

To the best of our knowledge no anti-microbial studies are done on these plants other than studies of its anti-oxidant properties [15]. Other species of *Erigeron* have been reported to have antifungal, antibacterial, antioxidant properties. *E. annuus* has been used in Chinese folk medicine for the treatment of indigestion, enteritis, epidemic hepatitis, and haematuria [16]. The chemical composition of the essential oils from *E. acris* and *E. annuus* is well known [17]. The antispasmodic, analgesic, sedative and anti-inflammatory effects of essential oils have been well established [18]. Therefore, *E. karvinskianus* can also be considered to possess great potential in harbouring immense medicinal properties. Hence this paper focuses on phytochemical screening, antifungal and antibacterial activity of various parts of *E. karvinskianus* (leaves, roots and stems) so as to investigate its antimicrobial activity.

2. Materials and methods

Petroleum ether, chloroform, methanol (99%) and ethyl acetate were purchased from merck, India. Potato dextrose agar medium, Nutrient Agar medium, Nutrient broth, Gentamicin antibiotic solution, Amphotericin B and antimycotic solution was purchased from Himedia, India.

2.1. Plant collection

Plant material (leaves, stems & roots) of *Erigeron karvinskianus* were collected from Kohima, in the vicinity of the Dzuvuru River, Nagaland. The botanical material was identified by Dr. N. Odyuo and deposited at Botanical survey of India, Eastern Regional Centre, Shillong, with voucher no. BSI/ERC/Tech/2022-23/176.

2.2. Extraction process

The leaves, stems and roots were washed with distilled water and air dried at room temperature for 3 weeks. The leaves were grounded separately from the stems and roots into uniform powder with the help of an electric grinder. 10 gm of the powder were used for soxhlet extraction with 300 ml of solvents (chloroform, methanol & water). The extraction with each solvent was performed at about 10 °C higher than the boiling point of the solvent, and the extraction was allowed to be carried out until the solvent in the extraction chamber became colourless. The extracts were filtered and solvents were evaporated using rotary evaporator. The dried extracts were stored in centrifuge tubes at 4°C for further use.

2.3. Phytochemical screening

The extracts were screened for the presence of secondary metabolites like, Alkaloids (Dragendroff test, Wagner's test), Carbohydrates (Benedict's test, Fehling's test), Cardiac glycosides (Keller-killiani test, Bromine water test), Flavonoids (Alkaline reagent test, Lead acetate test), Phenolic compounds (Iodine test, Ferric chloride test), Tannins (Braymer's test, 10% NaOH test), Phytosterols (Salkowski's test, Hesse's response), Terpenoids, Lignins (Labat test, Furfuraldehyde test) and Quinones (Alcoholic KOH test, Conc. HCl test). The detailed procedures of the test are given in supplementary table 1.

2.4. Test organism used in the study

Two species of bacteria and two species of fungi were taken into consideration for the antibacterial activity test and antifungal activity test respectively. *E.coli, S.aureus, A.niger and C. albicans* were purchased from MTCC, Chandigarh, India

2.5. Test for antibacterial and antifungal activity

The antifungal and antibacterial activities of the crude extracts were determined using agar well diffusion method. The antifungal and antibacterial agents present in the given sample were allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The resulting zones of inhibition will be uniformly circular as there will be a confluent lawn of growth. The diameters of zone of inhibition were measured in millimeters. For the antibacterial assay the medium was prepared by dissolving 2.8 g of the commercially available Nutrient Agar Medium in 100ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121 °C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm Petri plates (25-30ml/plate) while still molten. Nutrient broth was prepared by dissolving 2.8 g of commercially available nutrient medium in 100ml distilled water and boiled to dissolve the medium completely. The medium was dispensed as desired and sterilized by autoclaving at 15 lbs pressure (121 ºC) for 15 minutes. Petri plates containing 20 ml nutrient agar medium were seeded with 24hr culture of bacterial strains *(E.coli and S.aureus)* wells were cut and different concentration of samples (500 µg/ml, 250 µg/ml,100 µg/ml and 50 µg/ml) were added. The plates were then incubated at 37 °C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control.

For antifungal assay, potato dextrose agar medium was prepared by dissolving 40 gm of potato infusion, 4 gm of dextrose and 3.5 gm of agar in 200 ml of distilled water. The dissolved medium was autoclaved at 15 lbs pressure at 121°C for 15 minutes. The autoclaved medium was mixed well and poured onto 100mm petri plates (25-30 ml/plate) while still molten. Petri plates containing 20ml potato dextrose agar medium were seeded with 72hr culture of fungal strain *(A. niger and C. albicans)* wells were cut and different concentration of both the samples (500, 250,100 and 50 µg/ml) were added. The plates were then incubated at 37 °C for 48-72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around

the wells. Amphotericin B (100 units) was used as a positive control. The values for both the assays were calculated using Graph Pad Prism 6.0 software (USA).

3. Results and Discussion

The plant *Erigeron karvinskianus* was collected washed and parts like roots, stem and leaves were separated when still fresh. The separated plant parts were air dried at room temperature to avoid direct sunlight so as to prevent any unwanted photochemical reactions. After complete drying, the leaves and stem-root were grounded into uniform powder with the help of an electric grinder to enhance the rate of extraction. The powdered samples were subjected to Soxhlet extraction with four solvents *viz*., petroleum ether, chloroform, methanol and water keeping in mind their difference in polarity. The solvent petroleum ether was mainly used for the purpose of defatting the plant extract. The crude plant extracts obtained with the solvents were screened for the presence of phytochemicals. The result of the detailed phytochemical analysis of the leaves and stem-root has been provided in Table 1.

Table 1: Phytochemical analysis of extract of *Erigeron karvinskianus*

Sl. No.	Phytochemical test	Leaves extract			Stem-root extract		
		Chloroform	Methanol	Water	Chloroform	Methanol	Water
1.	**Detection of alkaloids**						
	(A) Dragendroff test	-	-	-	+	+	+
	(B) Wagner's test	-	-	-	-	-	-
2.	**Detection of carbohydrates**						
	(A) Molisch's test	-	+	-	-	-	-
	(B) Test for starch	-	-	-	-	-	-
3.	**Detection of reducing sugars**						
	(A) Benedict's test	-	+	+	-	-	-
	(B) Fehling's test	-	+	-	-	-	-
4.	**Detection of cardiac glycosides**						
	(A) Keller-Killani test	+	-	-	-	-	-
	(B) Bromine water test	-	-	-	-	-	-
5.	**Detection of flavonoids**						
	(A) Alkaline reagent test	-	+	+	+	+	+
	(B) Lead acetate test	-	+	+	+	+	+
6.	**Detection of phenolic compounds**						
	(A) Iodine test	-	+	-	-	-	-
	(B) Ferric chloride test	-	+	-	-	-	-
7.	**Detection of tannins**						
	(A) Braymer's test	-	+	+	-	-	-
	(B) 10% NaOH test	+	+	+	-	-	-
8.	**Detection of phytosterols**						
	(A) Salkowski's test	-	-	-	-	-	+
	(B) Hesse's response	-	-	-	-	-	-
9.	Detection of terpenoides	-	-	-	-	+	+
10.	**Detection of lignins**						
	(A) Labat test	+	-	-	-	-	-
	(B) Furfuraldehyde test	-	-	-	-	-	-
11.	**Detection of quinones**						
	(A) Alcoholic KOH test	-	-	-	-	-	-
	(B) Conc. HCl test	+	-	-	-	-	+

Supplementary Table 1: Procedure of the phytochemical tests performed

Test		Procedure	Observation (Indicating Positive Test)
Detection of alkaloids			
1.	(A) Dragendroff's test	Few ml of plant extract + 1-2ml Dragendroff's reagent	A reddish brown precipitate
	(B) Wagner's test	Few ml filtrate + 1-2 drops of Wagner's reagent (Along the sides of test tube)	A brown/reddish precipitate
Detection of Carbohydrates			
2.	(A) Molisch's test	2ml of plant extract + 2 drops of alcoholic α-naphthol + 1ml conc. H_2SO_4 (along the sides of test tube)	A violet ring
	(B) Test for starch	Few ml of plant extract + 5ml 5% KOH solution	A cinary colouration
Detection of reducing sugars			
3.	(A) Benedict's Test	0.5ml plant extract + 0.5ml Benedict's reagent + Boiled for 2 minutes	Green/yellow/red colour
	(B) Fehling's test	1ml each of Fehling's solution A & B + 1ml plant extract + boiled in water bath	A red precipitate
Detection of Cardiac Glycosides			
4.	(A) Keller-Killani test	1ml plant extract + 1.5ml glacial acetic acid + 1 drop of 5% ferric chloride + conc. H_2SO_4 (along the side of test tube)	A blue coloured solution (in acetic acid layer)
	(B) Bromine water test	Plant extract + few ml of bromine water	A yellow precipitate
Detection of Flavonoids			

5	(A) Alkaline reagent test	1ml plant extract + 2ml of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour, becomes colourless on addition of diluted acid
	(B) Lead acetate test	1ml of plant extract + few drops of 10% lead acetate solution	A yellow precipitate
Detection of Phenolic compounds			
6.	(A) Iodine test	1ml of plant extract + few drops of dil. Iodine solution	A transient red colour
	(B) Ferric Chloride test	Aqueous Plant extract + few drops 5% ferric chloride solution	Dark green/bluish black colour
Detection of Tannins			
7.	(A) Braymer's test	1ml plant extract + 3ml distilled water + 3 drops 10% Ferric chloride solution	Blue-green colour
	(B) 10% NaOH test	0.4ml plant extract + 4ml 10% NaOH + shaken well	Formation of emulsion
Detection of Phytosterols			
8.	(A) Salkowski's test	Plant extract + few drops of conc. H_2SO_4 (Shaken well and allowed to stand)	Red colour (in lower layer)
	(B) Hesse's response	5ml aqueous plant extract + 2ml chloroform + 2ml conc. H_2SO_4	Pink ring/Red colour (in lower chloroform layer)
Detection of Terpenoids			
9.		2ml of chloroform + 5ml plant extract, (evaporated on water bath) + 3ml conc. H_2SO_4 (boiled on water bath)	A grey coloured solution
Detection of Lignins			
10.	(A) Labat test	Plant extract solution + gallic acid	An olive green colour
	(B) Furfuraldehyde test	Plant extract solution + 2% furfuraldehyde solution	A red colour
Detection of Quinones			
11.	(A) Alcoholic KOH test	1ml plant extract + few ml alcoholic potassium hydroxide	Red to blue colour
	(B) Conc. HCl test	Plant extract + conc. HCl	A green colour

Supplementary Table 2: SD± Means of zone of inhibition obtained by aqueous and methanol extracts of leaves and stem-root against *E. coli* and *S. aureus*.

S. No	Name of the organism	Name of the test sample	Zone of Inhibition (mm) SD ± Mean				
			PC	500 µg/µl	250 µg/µl	100 µg/µl	50 µg/µl
1.	*E.coli*	Methanol extract (leaves)	18.5±0.7	19.5±0.7	14.5±0.7	13.5±0.7	12.5±0.7
2.	*S.aureus*		16.5±0.7	16±1.04	13.5±0.7	12.5±0.7	0
1.	*E.coli*	Aqueous extract (leaves)	18.5±0.7	14.5±0.7	13.5±0.7	12.5±0.7	8±1.4
2.	*S.aureus*		16.5±0.7	12.5±0.7	11.5±0.7	0	0
1.	*E. coli*	Aqueous extract (stem-root)	24±1.4	18.5±0.7	16.5±0.7	14±1.4	11.5±0.7
2.	*S.aureus*		24.5±0.7	17.5±0.7	14.5±0.7	10.5±0.7	11±1.4
1.	*E. coli*	Methanol extract (stem-root)	23±0	13.5±0.7	12.5±0.7	10.5±0.7	8±1.4
2.	*S. aureus*		24.5±0.7	12±1.4	11.5±0.7	9.5±0.7	7.5±0.7

Supplementary table 3: SD± Means of zone of inhibition obtained by aqueous and methanol extract of leaves and stem-root against *A.niger and C.albicans.*

S. No	Name of the organism	Name of the test sample	Zone of inhibition (mm) SD ± Mean				
			PC	500 µg/µl	250 µg/µl	100 µg/µl	50 µg/µl
1.	*A.niger*	Aqueous extract (leaves)	16.5±0.7	14.5±0.7	13±0	0	0
2.	*C.albicans*		17.5±0.7	12.5±0.7	10.5±0.7	9.5±0.7	0
1.	*A.niger*	Methanol extract (leaves)	17.5±0.7	14.5±0.7	13.5±0.7	12±0	11±0
2.	*C.albicans*		18.5±0.7	16±0	13.5±0.5	12±0	10.5±0.7
1.	*A. niger*	Aqueous extract (stem-root)	10±0	10.5±0.7	9±0	8.5±0.7	7±0
2.	*C. albicans*		12.5±0.7	12.5±0.7	11±0	9±0	7±0
1.	*A. niger*	Methanol extract (stem-root)	10.5±0.7	9±0	5.5±0.7	4.5±0.7	0
2.	*C. albicans*		12±1.4	9±0	6±0	5±0	0

The phytochemical analysis of the leaf extract in chloroform solvent showed the presence of glycosides, tannins, lignins and quinones. Other important metabolites like alkaloids, flavonoids, phenolic compounds were absent; hence the chloroform extract was not subjected to further analysis for anti-microbial activity. The phytochemical screening of the stem-root extract showed the presence of alkaloids and flavonoids in the chloroform extract however other metabolites were absent. Screening of methanol and aqueous solvent of leaves extract showed the presence of important phytochemicals such as flavonoids, phenolic compound and tannins and therefore was subjected for further antimicrobial activity analysis. Also that of stem-root extracts contained alkaloids, flavonoids, terpenoids and quinones

Crude methanol and aqueous extracts of the leaves and stem-roots of *E. karvinskianus* were analysed for antifungal and antibacterial activities and all the results are presented in supplementary figure 1 & 2. *Aspergillus niger*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* were the test organisms considered for the antifungal and antibacterial arrays. The graphical representation of the antimicrobial activities for the leaves, stem and roots is provided in figure 1.

Supplementary figure 1. Antibacterial activity of crude extracts of leaves and stem-root of *E. karvinskianus*.

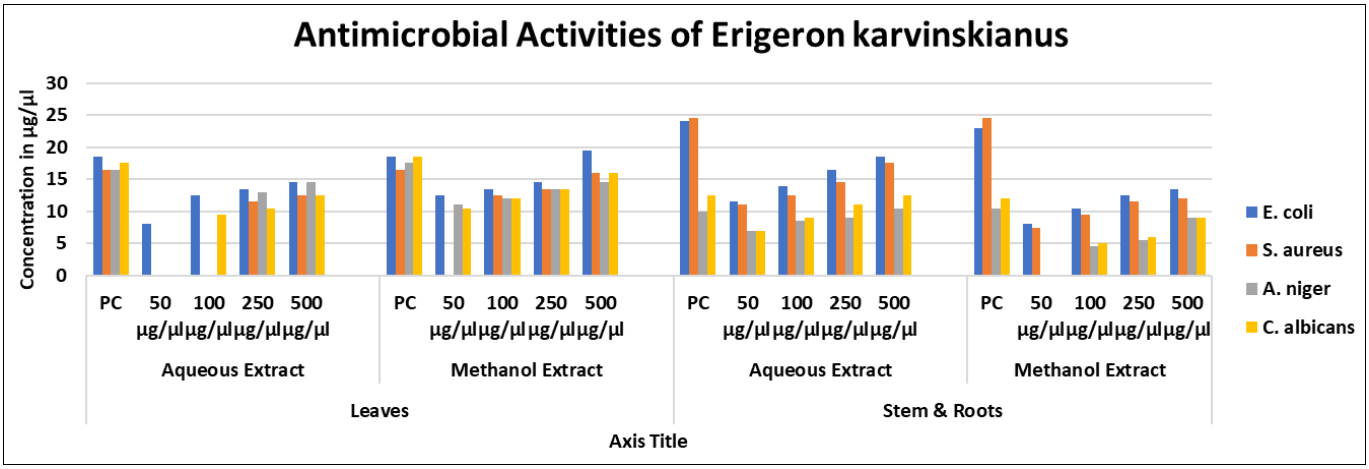

Fig 1: Antimicrobial activities of *Erigeron karvinskianus*: aqueous and methanolic extract of leaves and stem-root.

Supplementary figure 1. Antibacterial activity of crude extracts of leaves and stem-root of *E. karvinskianus*.

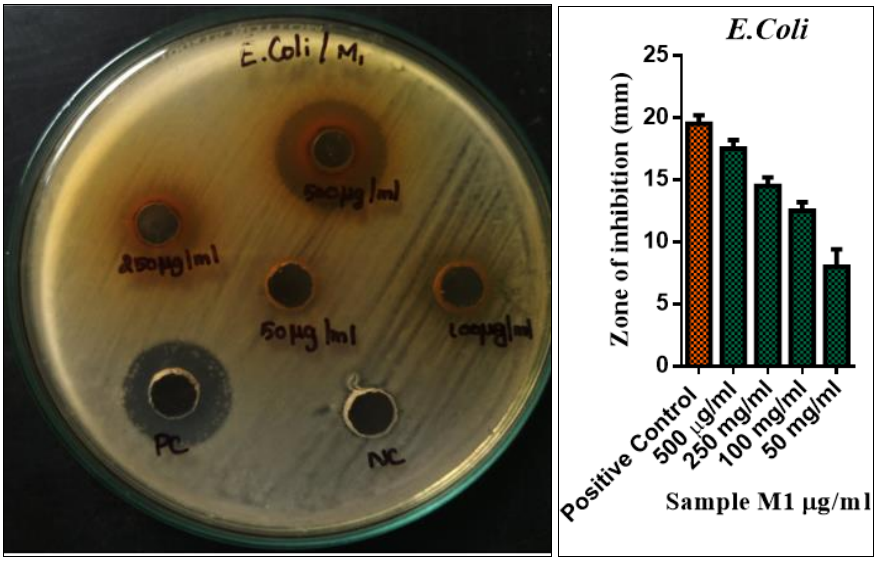

Fig 1(a): Antibacterial activity of methanol leaves extract of *E. karvinskianus* against *E.coli*.

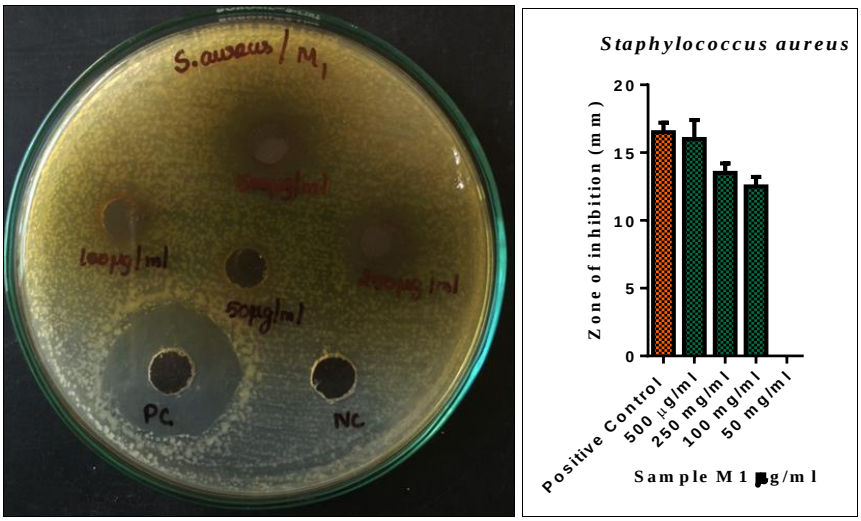

Fig 1(b): Antibacterial activity of crude methanol leaves extract of *E. karvinskianus* against *S. aureus*

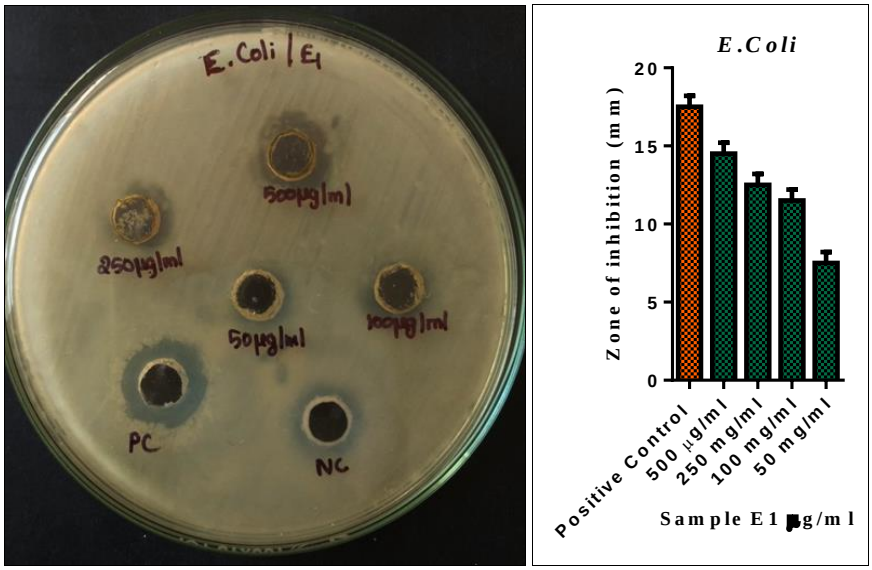

Figure 1(c): Antibacterial activity of crude aqueous leaves extract of *E. karvinskianus* against *E. coli*.

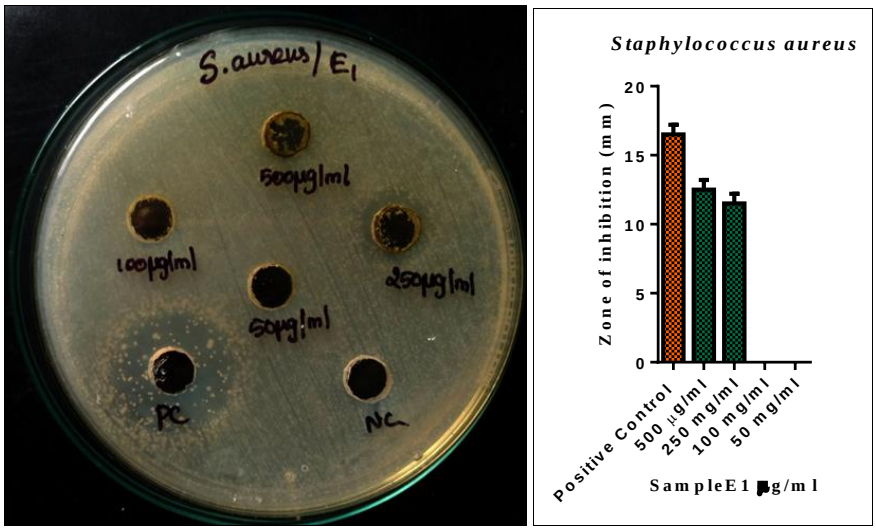

Fig 1(d): Antibacterial activity of crude aqueous leaves extract of *E. karvinskianus* against *S. aureus*.

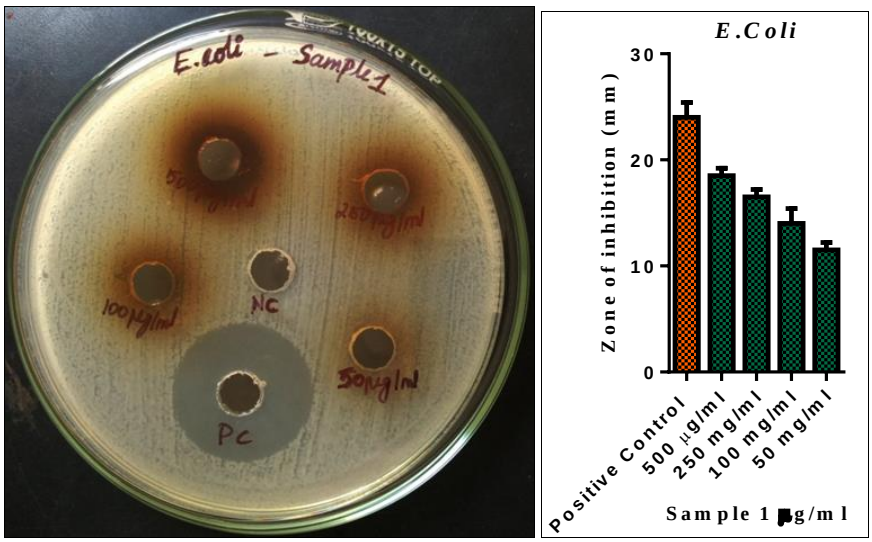

Fig 1(e): Antibacterial activity of crude aqueous stem-root extracts of *E. karvinskianus* against *E. coli*.

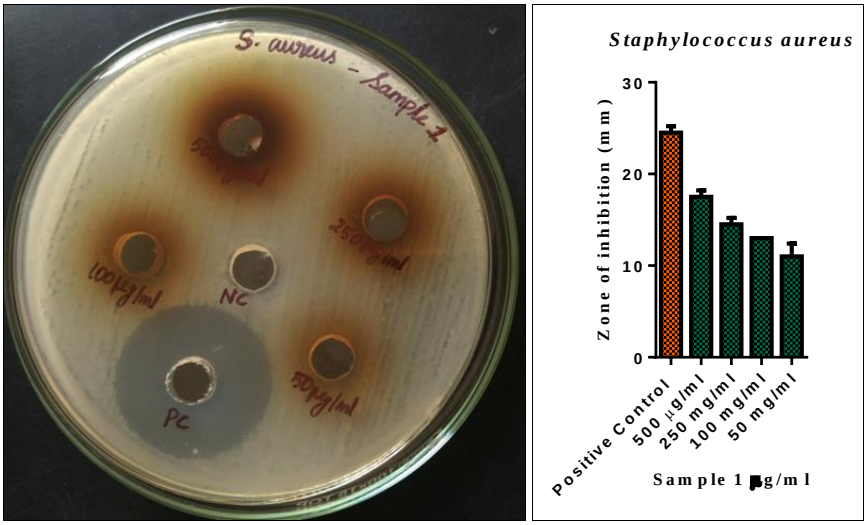

Fig 1(f): Antibacterial activity of crude aqueous stem-root extract of *E. karvinskianus* against *S. aureus*.

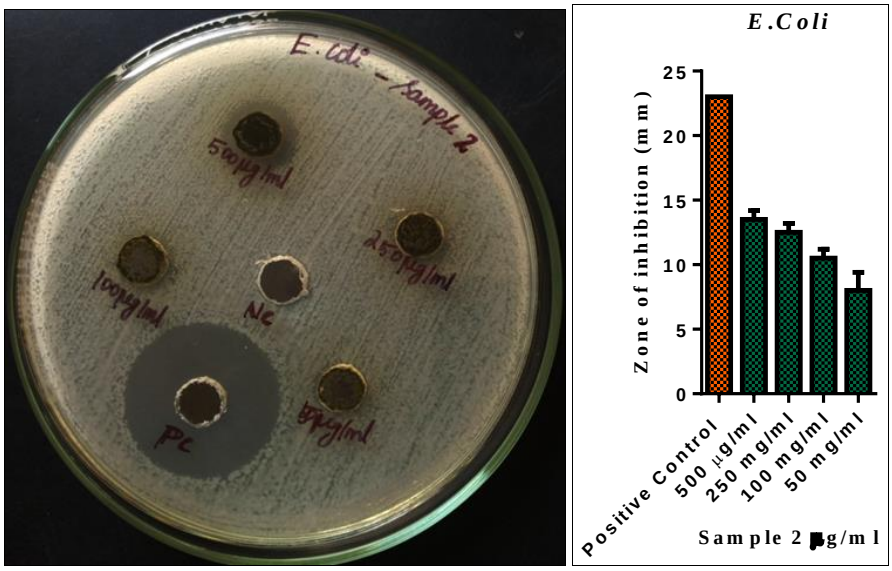

Fig 1(g): Antibacterial activity of crude methanol stem-root extract of *E. karvinskianus against E. coli*.

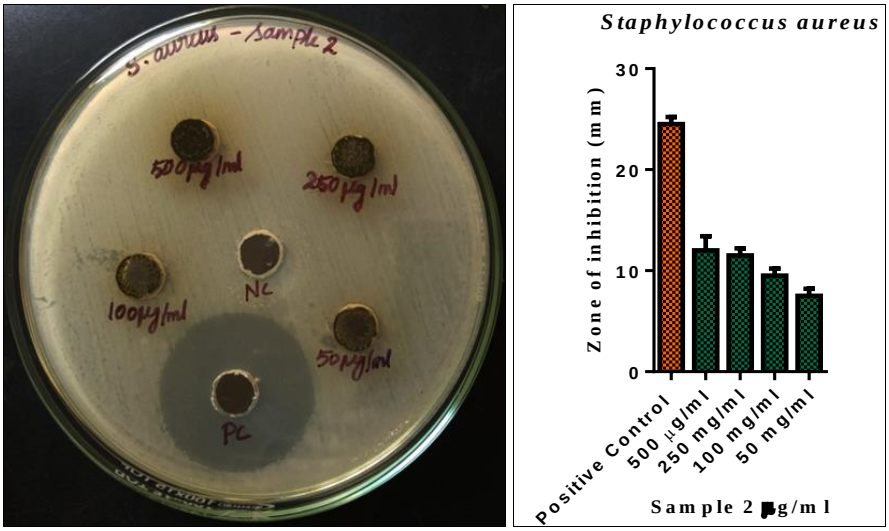

Fig 1(h): Antibacterial activity of crude methanol stem-root extract of *E. karvinskianus* against *S. aureus*.

In the graph, the X-axis corresponds to various concentrations such as 50µg/µl, 100µg/µl, 250µg/µl and 500µg/µl of the extract considered for the analysis where all the test organisms are depicted with different colours- *E. coli* represented by blue colour, *S. aureus* by brown colour, *A. niger* by gray colour and *C. albicans* by yellow colour. The Y-axis expresses the antifungal and antibacterial activity i.e., zones of inhibition with respect to the positive control used in the study. The graph for both the leaves extract and stem-root extract in methanol and aqueous solvent showed similar trend of increasing anti-microbial activity with increase in the concentration of the extracts. Besides aqueous extract of

stem-root, which showed some considerable level of anti-microbial activity against all the four test organisms at the concentration of 50µg/µl, the other extracts namely aqueous extract of leaves showed no activity against *S. aureus*, *A. niger* and *C. albicans* at 50µg/µl concentration; methanol extract of leaves showed zero activity against *S. aureus* and methanol extract of stem-roots exhibited no activity against *A. niger* and *C. albicans* at 50µg/µl. At higher concentration of 500µg/µl, the aqueous extract of stem-root showed promising antifungal activity with zones of inhibition measuring 10.5mm against *A. niger* which was more than that of the positive control of 10mm (Figure 2A) and 12.5mm against *C. albicans* which was equal to the positive control at the same concentration (Figure 2B). In the methanol extract at 500µg/µl concentration the zone of inhibition was recorded to be 19.5mm against *E. coli* which was significantly higher than the positive control. However, no such activity was seen in the case of antifungal activity in the leaf extracts. These significant results may be due to the presence of phytochemicals such as flavonoids, phenolic compounds and tannins that were detected in the phytochemical screening of plant extracts in methanol and aqueous solvents (Figure 2C).

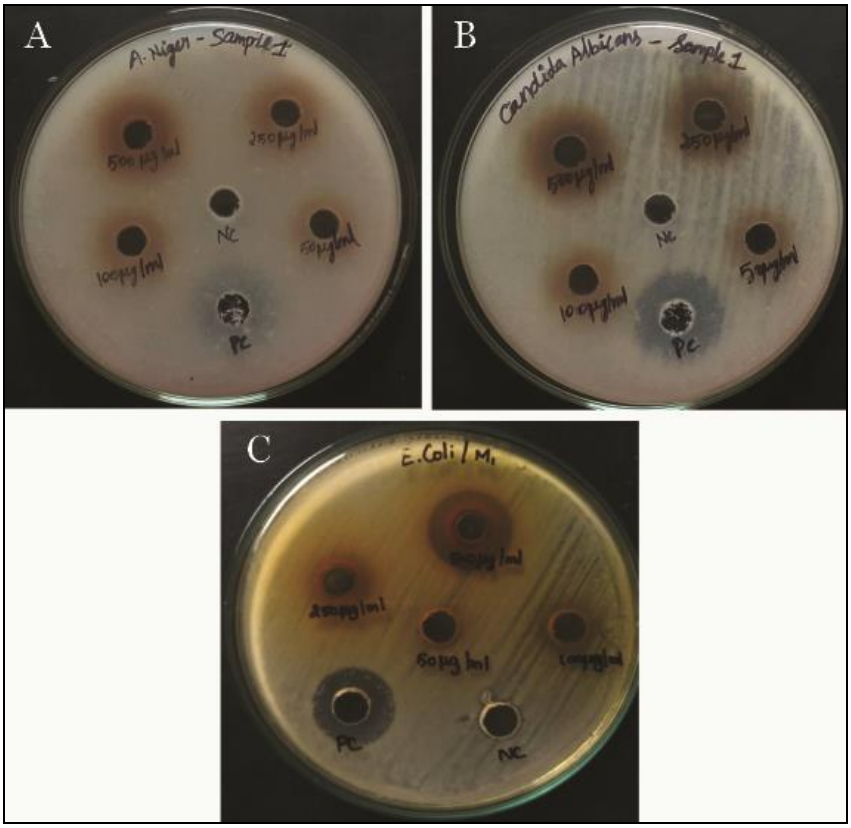

Fig 2: (A) Antifungal activity of aqueous extract of stem-root against *Aspergillus niger*, (B) Antifungal activity of aqueous extract of stem-root against *Candida albicans*, (C) Antibacterial activity of methanol extract of leaves against *Escherichia coli*.

Supplementary figure 2. Antifungal activity of crude extracts of leaves and stem-roots of *E. karvinskianus*.

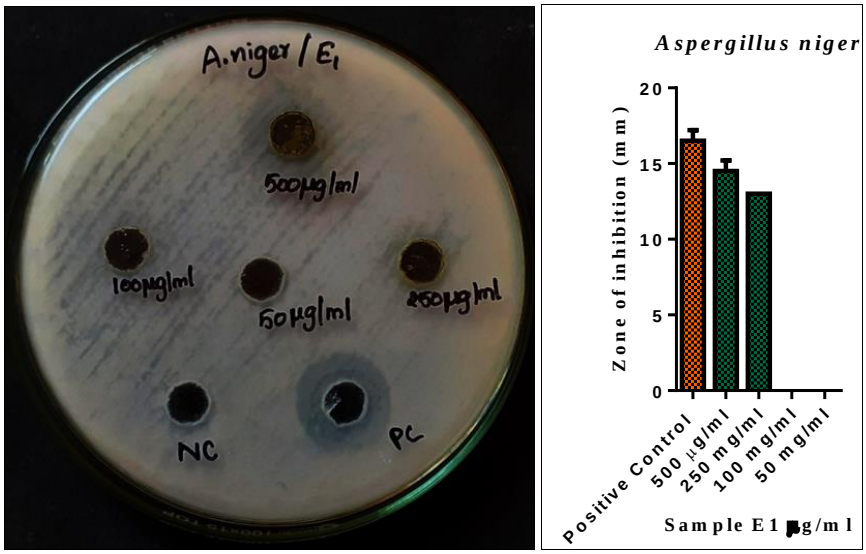

Fig 2(a): Antifungal activity of crude aqueous leaves extract of *E. karvinskianus* against *A. niger*.

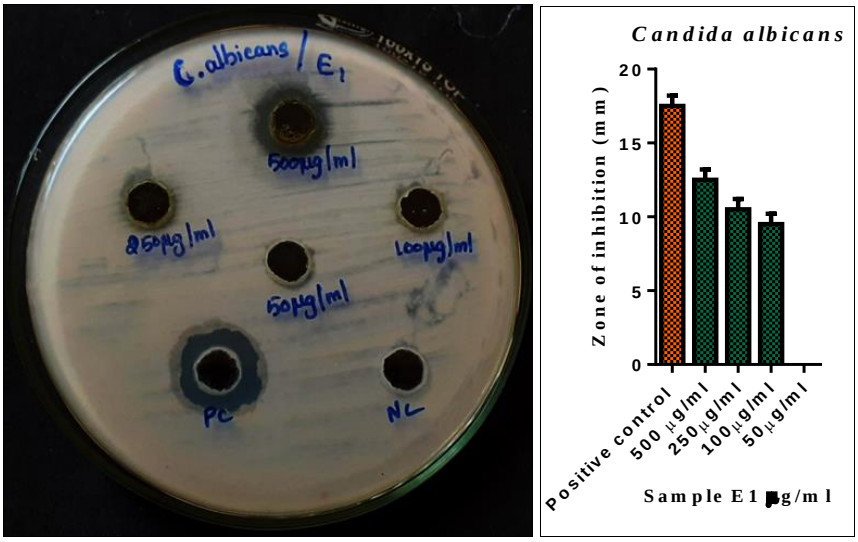

Fig 2(b): Antifungal activity of crude aqueous leaves extract of *E. karvinskianus* against *C. albicans.*

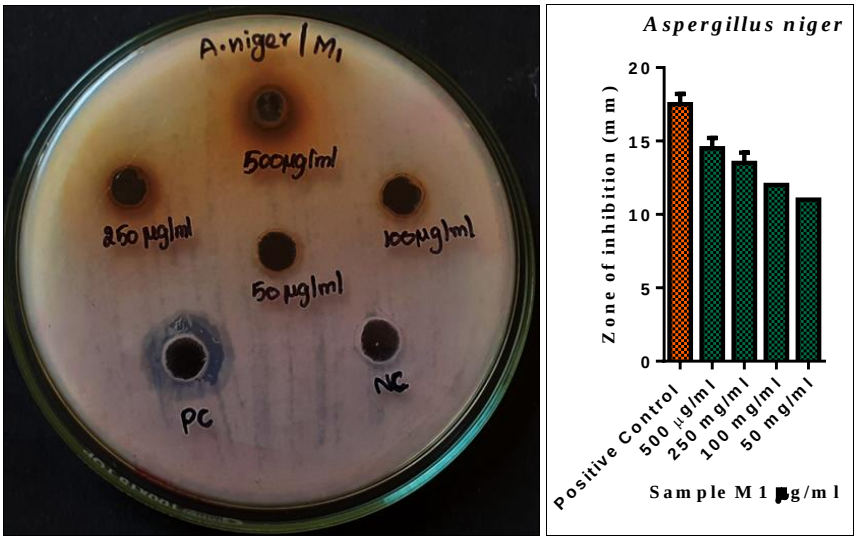

Fig 2(c): Antifungal activity of crude methanol leaves extract of *E. karvinskianus* against *A. niger*.

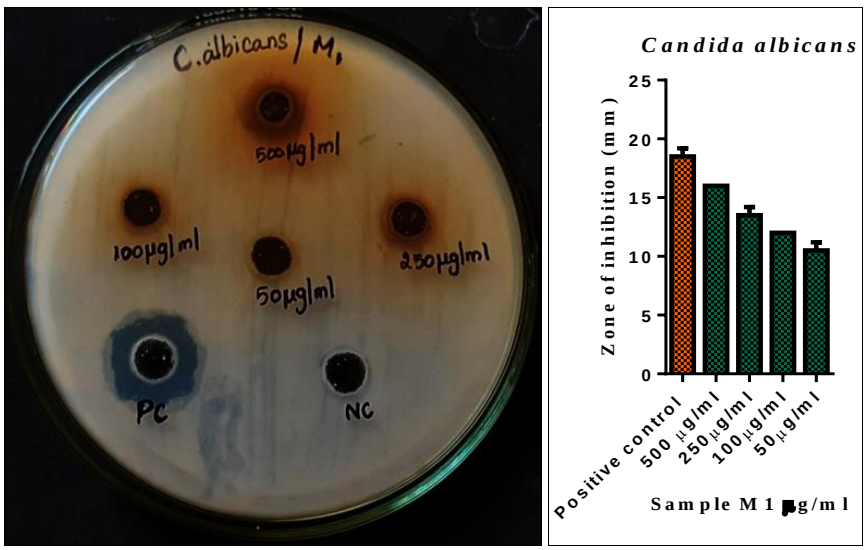

Fig 2(d): Antifungal activity of crude methanol leaves extract of *E. karvinskianus* against *C. albicans*.

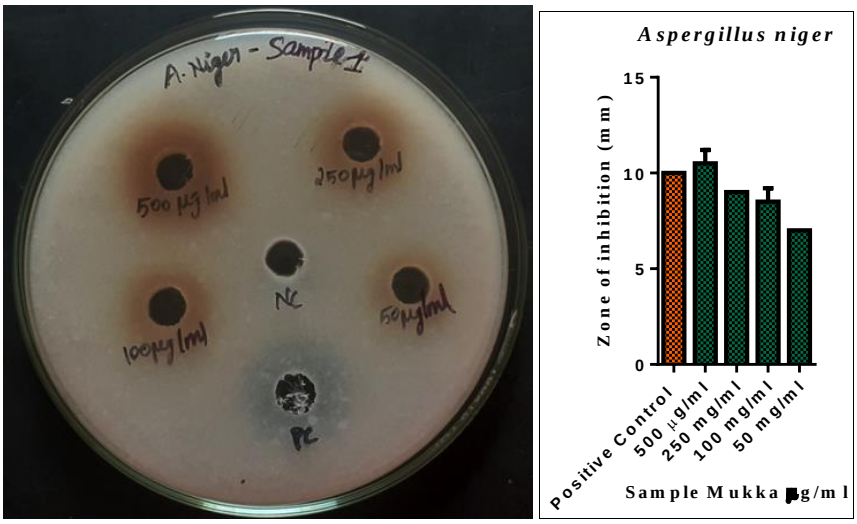

Fig 2(e): Antifungal activity of crude aqueous stem-root extract of *E.karvinskianus* against *A. niger*.

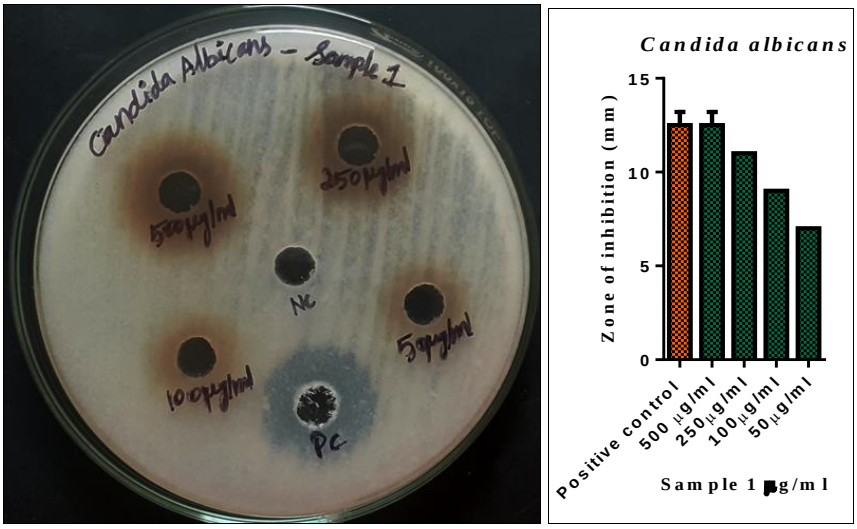

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Fig 2(f): Antifungal activity of crude aqueous stem-root extract of *E.karvinskianus* against *C. albicans*.

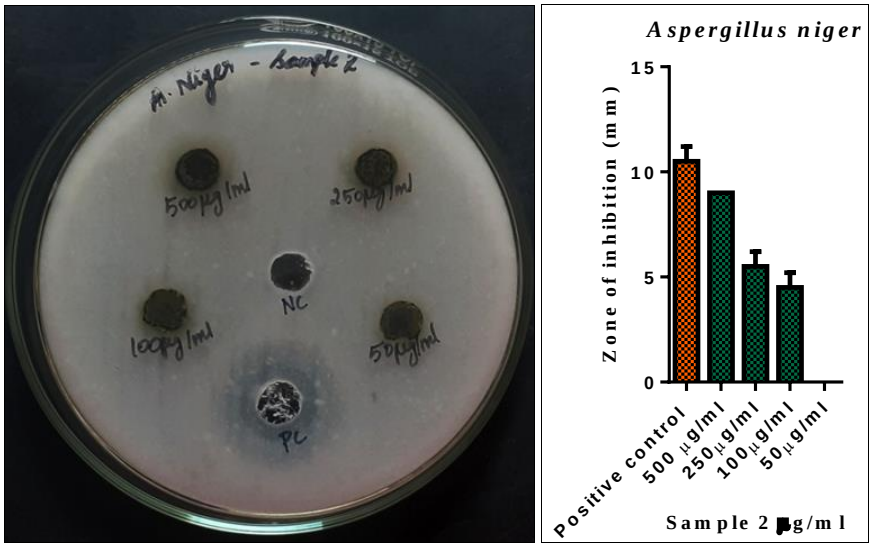

Fig 2(g): Antifungal activity of crude methanol stem-root extract of *E. karvinskianus* against *A. niger*.

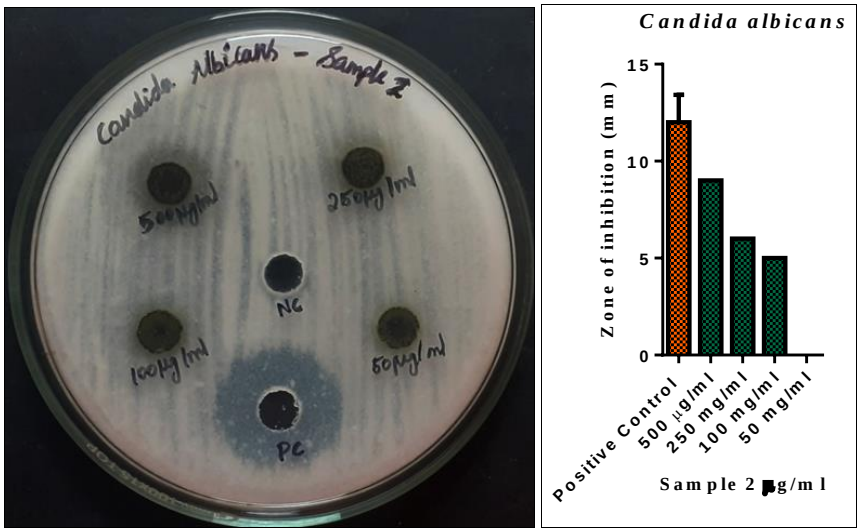

Fig 2(h): Antifungal activity of crude methanol stem-roots extract of *E. karvinskianus* against *C. albicans*.

4. Conclusion

The plant specimen *Erigeron karvinskianus* was collected separated into different parts namely leaves, roots and stem and air dried. They were then subjected to Soxhlet extraction using water, methanol and chloroform as the solvents. Phytochemical analysis or screening showed the presence of important phytoconstituents in methanol and aqueous extract, as a result further antifungal and antibacterial activity tests were done using the crude extracts against two test organisms each of fungi and bacteria *viz*., *A. niger*, *C. albicans*, *E.coli* and *S. aureus* respectively. The analysis was carried out using Amphotericin B as positive control against fungi and gentamicin as positive control against bacteria. The methanol extract of leaves and the aqueous extract of stem-roots showed promising results against microbial activities. Specifically, antibacterial activity against *E.coli* in methanol extract and antifungal activity against *A. niger* and *C. albicans* where they exhibited zones of inhibition greater than the positive control used for each of them. Therefore, these promising results opens prospects for further analysis such as isolation and characterization of the phytoconstituents as well as further tests like GCMS, LCMS etc. Also detailed antimicrobial activities of *E. karvinskianus* can be further studied extensively using more microbial species as test organisms in the future.

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6. Conflict of Interest

No potential conflict of interest was reported by the authors.

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