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Antimicrobial activity and phytochemical screening of whole plant extracts of *pholidota articulate* lindl

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Abstract

Pholidota articulata is a clump-forming epiphyte or lithophyte with crowded pseudobulbs called the necklace orchid or common rattlesnake orchid. This study used extracts from the entire *Pholidota articulata* plant to identify phytochemical constituents and antibacterial activity. This orchid's different solvent extracts, including methanol, ethanol, hexane, and chloroform, were tested for their in vitro antimicrobial activity against three fungi (Penicillium sp., Rhizopus sp., and Aspergillus niger) and five clinical pathogenic bacteria (Staphylococcus aureus, Bacillus subtilis, Vibrio cholerae, Escherichia coli, and Klebsiella pneumonia) using the disc diffusion method. A preliminary phytochemical examination was carried out to find alkaloids, terpenoids, flavonoids, phenols, tannins, steroids, and glycosides, among other substances. Every extract exhibited varying inhibitory potential against the microorganisms tested for this investigation. The maximum inhibition zone (14–15 mm) was discovered at a concentration of 10.0 mg-1 of chloroform extract, demonstrating antibacterial activity against all bacteria with a zone of inhibition spanning 5–15 mm. Aspergillus niger, Rhizopus sp., and Penicillium sp. were all significantly inhibited by chloroform extract, with the largest zone of inhibition (16–17 mm). We successfully identified antimicrobial activity that might be used to isolate and characterize the impact of new phytochemicals on several infectious disorders, mainly because of the rise of antimicrobial agents and drug-resistant microbes.

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INTRODUCTION

The orchid family's Pholidota articulata, also called the common rattlesnake orchid or necklace orchid, is a clump-forming epiphyte or lithophyte with densely packed pseudobulbs. Up to sixty white, cream-colored, or greenish cup-shaped blooms are arranged in two ranks along a wiry flowering stalk with a single pleated, leathery leaf per pseudobulb. A vast, papery bract is at the base of each flower [1]. This species is indigenous from tropical and subtropical Asia to the southwestern

Pacific. An epiphytic or lithophytic herb that forms clumps, *Pholidota articulata,* has dense pseudobulbs that are 80–120 mm (3.1–4.7 in) long and 30–50 mm (1.2–2.0 in) broad. Articulate Lindl. Pholidota. Paste that is applied to broken bones and ingested as a tonic. Cancer is treated using root powder. Berries cure wounds, severe injuries, ulcers, and skin outbreaks. Reduces edema and eliminates gas. Additionally, it is used to treat uterine prolapse, headaches, dizziness, irregular menstruation, and coughing brought on by body heat [2].

Using novel compounds not derived from pre-existing synthetic antimicrobial drugs is one strategy to stop pathogenic species from developing antibiotic resistance. The need to look for antibacterial chemicals as a substitute arose from medication resistance in human diseases. Consequently, screening medicinal plants is essential to resolving these new issues. Conversely, antimicrobial compounds generated from plants have no known adverse effects and may be used therapeutically to treat various infectious disorders. Despite significant advancements in medical research and treatment over the 20th century, infectious diseases continue to rank among the important causes of mortality globally, killing nearly 60,000 people every day [3]. Finding novel antibacterial compounds from other sources, such as plants, has become necessary in the current situation of multiple drug resistance to human pathogenic pathogens. Plants use phytochemicals, also known as secondary metabolites, chemical molecules produced during regular metabolic processes, as a defense mechanism. Despite the belief that plant-based medications are completely safe and devoid of side effects, phototherapeutic substances are less likely to have incorrect or adverse side effects than synthetic medications. Interest in finding novel antibacterial and antioxidant chemicals from nature has increased due to infections' tolerance to antibiotics and the oxidative stress of free radicals. Plant-derived natural crude drug extracts can be abundant sources of these new drugs. Thus, the phytochemical analysis and antibacterial activity of *Pholidota articulata* were assessed in this study in light of its therapeutic qualities. This plant species' therapeutic potential would be confirmed, opening the door for more research on the plant species and assisting the scientific community in developing strategies to protect this precious, vulnerable medicinal plant species [4].

MATERIALS AND METHODS

Materials:

Pholidota articulata, an epiphytic orchid, was gathered as plant material from Tirupati, Andhra Pradesh, India. For this purpose, the entire plant was utilized as plant material. Analytical-grade solvents, including methanol, ethanol, chloroform, and hexane, were purchased commercially from Merck Limited in Mumbai, India.

Preparation of Extract:

After washing various P. articulate portions with running tap water and absorbing the excess water on tissue paper, the parts were diced into minute pieces and dried in an incubator set at 28ºC (**Figure 1** A, B). A pulverizer was then used to pulverize them coarsely (**Figure 1** C). Then, using the sonication process, the coarse particles were successively extracted using organic solvents such as methanol, ethanol, chloroform, and hexane. Ultrasound with frequencies between 20 and 2000 kHz is used in the operation; this causes cavitations and enhances the permeability of cell walls [5]. After that, the extracts were gathered and distilled out on a water bath at atmospheric pressure, and any remaining solvent residue was eliminated in a vacuum before being kept at 4ºC (**Figure 1**D). Then, it is the study of phytochemicals and antibacterial activity. The proper amount is weighed and mixed with DMSO to create a stock solution of 10 mg/ml of each extract (crude drug) (**Figure 1**E). After sterilizing the stock solution with a 0.2 µm pyrogenic filter, DMSO was used to dilute it further to achieve concentrations of 5 mg/ml, 2.5 mg/ml, and 1.25 mg/ml. Extract concentrations were made using the filter paper disc method; 5 mm diameter discs made with No. 1 Whatman filter paper were autoclave sterilized. After that, various extract concentrations were used to coat the discs [6].

Test of Microorganisms:

The Popular Diagnostic Centre (Pvt.) Ltd. provided clinical strains of bacteria that were isolated from patient samples. Gram-positive (Staphylococcus aureus and Bacillus subtilis) and gram-negative (Vibrio cholerae, Escherichia coli, and Klebsiella

pneumonia) were the five bacteria examined in this study. These bacterial cultures were cultivated for 24 hours at 37°C in a nutrient broth (liquid media). These bacteria were kept in a pure culture on a nutrient agar medium at 4°C. Aspergillus niger, Rhizopus sp., and Penicillium sp. were the fungal species used in this investigation [7].

Antibacterial Activity:

The antibacterial activity of the organisms was shown in cultures that were 24 hours old. 15 cc of nutrient agar media was poured into 90 cm sterile Petri dishes to create the nutrient agar medium plates. After five minutes of solidification, the dishes were infected with 0.1% inoculum.No. 1 Whatman filter paper was used to prepare the 5 mm diameter agar disc diffusion method and was autoclave sterilized. After that, different concentrations of the plant extracts were deposited onto the discs. After that, the plates were incubated for 24 hours at 37°C. The study's control for the bacteria above was ampicillin (10 mg/ml) [8].

Antifungal Activity:

The extracts of P. articulata plants were examined for antifungal activity using the agar disc diffusion method. 48-hour-old fungal cultures cultivated on potato dextrose agar (PDA) were utilized to inoculate fungal strains on PDA plates. Melted PDA was mixed with an aliquot (0.02 ml) of inoculums and then transferred into 90 cm petri plates. Following solidification, a cork borer was used to create the proper wells on the agar plate [9]. The agar well diffusion method added several extract concentrations to the medium. The antifungal activity of plant extracts was observed during a 24- to 48-hour incubation period at 28ºC. Zones of fungal growth inhibition around the plant extracts were measured to assess the antifungal activity. The antibiotic zone scale was used to measure the inhibition zones' diameter in millimeters. The experiment was conducted three times; the inhibition zones were noted, and the average antifungal activity values were computed. The control for fungi was ketoconazole (10 mg/ml).

Phytochemical Analysis:

Various solvent extracts were used to conduct phytochemical assays, detailed below. Regular qualitative chemical analyses of methanol, ethanol, chloroform, and hexane were performed per conventional protocols to determine the nature of the phytochemical constituents [10].

Different Phytoconstituents Tested Under This Study

Terpenoids:

To create a layer, 5 milliliters of the extract were combined with 2 milliliters of chloroform and concentrated sulphuric acid. Terpenoids were indicated by a reddish-brown coloring in the interface.

Flavonoids: concentrated sulphuric acid was added after a part of the aqueous extract was added to 5 milliliters of the diluted ammonia solution. A yellow appearance indicates the presence of flavonoids.

Reducing sugars: The presence of reducing sugar is indicated by the production of a reddish-orange color when 2 milliliters of Fehling's reagent A (or B) and 2 milliliters of water were added to the test solution.

Phenols: Mix two milliliters of ferric chloride solution with two millilitres of plant extract to test for phenols. The solution's bluish-green appearance indicates the presence of phenols.

Alkaloids: Five milliliters of extract and five milliliters of 2N HCl are combined, boiled, and filtered. The filtrate receives a few drops of Mayer's reagent.

The formation of a cream-colored precipitate was a rapid indicator of the presence of alkaloids. Five milliliters of extracts are heated in ten milliliters of distilled water in a test tube, and the mixture is then quickly agitated for around thirty seconds to check for saponins. The test tube is let to settle for half an hour. The production of foam indicates the presence of saponins [11].

Tannins: A few drops of 1% lead acetate were added to five milliliters of plant extract. The development of a yellow precipitate indicates the presence of tannins.

Steroids: One milliliter of the extract was diluted in ten milliliters of chloroform, and an equivalent volume of concentrated sulphuric acid was introduced from the test tube walls to test for the presence of steroids. The presence of steroids is

indicated by the upper layer becoming red and the fluorescence turning yellow with green.

Amino acids: One milliliter of the extract was mixed with a few drops of the Ninhydrin reagent. A purple color indicates the presence of amino acids.

Glycosides: Chloroform was added along the sides of 1 milliliter of the extract and 1 milliliter of alpha naphthol, and the color development was seen and noted. Glycosides are present when a violet color develops.

Carbohydrates: After shaking a 10% aqueous solution of alpha naphtali and adding concentrated H2SO4 along the tube's side, an alcoholic solution of the drug was added. Carbohydrates are indicated as a violet ring at the intersection of two liquids [12].

RESULTS

Both gram-positive and gram-negative bacteria were shown to be susceptible to the antibacterial effects of several solvents of whole plant extracts of Pholidota articulata. The antibacterial sensitivity of different solvent extracts was lowest (5.10 - 6.10 mm) with ethanol at a concentration of 1.25 ml-1 and highest (8.20 - 15.40 mm) with a chloroform extract of whole *Pholidota articulata* plants. Each sample's inhibition zone diameter was compared to a common antibiotic's (**Figure 2**). All of the harmful bacteria (E. coli: 13.10 mm, V. cholera: 8.20 mm, S. aureus: 15.40 mm, B. subtilis: 11.60 mm, and K. pneumonia: 14.10 mm; **Figure 1**) were most inhibited by the chloroform extracts of P. articulata. However, compared to other P. articulata extracts, the hexane extract displays the second-highest zone (8.10 - 9.60 mm) (**Table 1**). Methanol, ethanol, chloroform, and hexane extracts showed antimitotic action against the tested fungal isolates (Penicillium sp., Rhizopus sp., and Aspergillus niger) in another antimicrobial activity assay. When P. articulata whole plant extract was used to test for these fungi's susceptibility, the results were noteworthy because most of these fungi have recently been linked to immune-compromised patients who frequently get opportunistic infections. Aspergillus niger, Rhizopus sp., and Penicillium sp. were all most active against the chloroform extract, with inhibition zones of 17.20 mm, 16.10 mm, and 16.70 mm, respectively. Methanol and ethanol extract came next, while hexane extract came last (**Table 2**). The results of phytochemical activities demonstrated that the whole plant extracts of P. Articulata contained bioactive substances such as alkaloids, terpenoids, flavonoids, phenols, tannins, steroids, and glycosides. All P. articulata extract types contained alkaloids and glycosides, but none of the plant extracts contained carbohydrates, amino acids, saponins, or reducing sugar (**Table 3**).

DISCUSSION

The methodology section briefly describes the antimicrobial investigations carried out to examine the antibacterial effectiveness of P. articulata extracts against a few chosen species of bacteria and fungi. While the other three extracts were shown to be less potent against the examined organisms, the current study demonstrated that P. articulata whole plant extracts in chloroform gave the largest zone of inhibition (**Table 1** and **Table 2**). The variation is explained by the active ingredient's solubility in various solvents. However, compared to the extract of Vanda coerulea leaves (2.6 mm and 4.4 mm), the chloroform extract of P. articulata leaves showed more significant inhibition against S. aureus and E. coli (11.00 mm and 11.00 mm). Because of its broad-spectrum antibacterial activity, P. articulata is a possibility for bioprospecting for antibiotic and antifungal medications. The potential of chemicals against the common strains may be investigated to create treatments for microorganisms. Alkaloids, flavonoids, terpenoids, glycosides, steroids, tannins, and phenols were all detected in the plant extracts. Alkaloids, tannins, and saponins increased antibacterial action against harmful microbes. It is well known that the secondary metabolites of many chemical types found in plant species have antibacterial properties. Likely, flavonoids' capacity to form complexes with soluble and extracellular proteins and the bacterial cell wall makes them excellent antibacterial agents against various pathogens; more lipophilic flavonoids may also cause microbial membrane disruption. It is well known that the phenols and polyphenols found in plants are harmful to microbes.Furthermore, it is generally known that higher plants contain antibacterial chemicals, which have served as a source of inspiration for new therapeutic

Table 1 Four distinct solvent extracts of Pholidota articulates inhibition zone against bacteria

Solvent Extracts	Conc. (mgl-1)	Inhibition zone diameter (mm ± S.E)				
		Bacteria				
		Gram (+)		Gram (-)		
		Staphylococcus aureus	Bacillus subtilis	Vibrio cholerae	Escherichia coli	Klebsiella pneumonia
Control	10.0	21.62 ± 0.38	21.82 ±0.77	23.00 ± 0.77	26.21±0.47	21.42 ± 0.66
Methanol	10.0	9.21 ± 0.23	8.00 ± 0.27	8.31 ± 0.37	9.00± 0.33	9.41 ± 0.30
	6.00	9.61± 0.28	9.81 ± 0.35	7.21 ± 0.36	7.41 ± 0.29	7.62 ± 0.24
	2.50	8.00 ± 0.19	8.00 ± 0.33	5.63 ± 0.19	-	5.21 ± 0.35
	1.26	-	-	-	-	-
Ethanol	10.0	7.36 ± 0.32	6.21 ± 0.32	8.11 ± 0.33	9.31 ± 0.32	7.51 ± 0.27
	5.00	6.61 ± 0.29	5.26 ± 0.17	6.31 ± 0.32	7.26 ± 0.29	-
	2.51	-	5.21 ± 0.35	5.11 ± 0.12	7.11 ± 0.22	-
	1.26	-	-	-	-	-
Chloroform	10.0	16.41 ± 0.42	11.61± 0.28	8.21 ± 0.29	14.11±0.38	15.11 ± 0.33
	5.00	15.71 ± 0.57	12.21± 0.22	7.41 ± 0.15	13.21±0.43	14.31 ± 0.39
	2.50	14.31 ± 0.39	6.21± 0.23	-	12.41±0.30	13.51 ± 0.42
	1.25	13.61 ± 0.40	-	-	11.62±0.38	-
Hexane	10.0	8.61 ± 0.38	8.31 ± 0.23	-	8.11 ± 0.18	8.61 ± 0.32
	5.00	7.71 ± 0.18	8.27 ± 0.15	-	6.21 ± 0.22	8.41 ± 0.22
	2.51	-	6.31 ± 0.34	-	6.00 ± 0.15	6.51 ± 0.25
	1.26	-	-	-	-	-

Table 2 Zone of inhibition of Pholidota articulate against three fungi using four distinct solvent extracts

Fungal organisms	Inhibition zone diameter in mm					
	Different solvent extract					
	Cont. 1	Cont. 2	Methanol	Ethanol	Chloroform	Hexane
Penicillium sp	-	24.00 ± 0.00	13.40 ± 0.26	12.50 ± 0.39	16.21 ± 0.35	14.00 ± 0.20
Rhizopus sp.	-	22.00 ± 0.00	14.50 ± 0.29	14.10 ± 0.32	16.10 ± 0.19	13.30 ± 0.38
Aspergillus niger	-	20.00 ± 0.00	15.90 ± 0.21	13.40 ± 0.36	16.70 ± 0.34	13.00 ± 0.27

[Cont. 1 = Ketoconazole (10 mg/ml) as a positive control, Cont. 2 = Distilled water as a negative control, - = No result]

Table 3 Preliminary phytochemical examination of various Pholidota articulata extracts

Phytoconstituents	Solvent Extracts			
	Methanol	Ethanol	Chloroform	Hexane
Terpenoids	+	+	+	-
Flavonoids	+	+	-	-
Reducing sugars	-	-	-	-
Phenols	+	-	-	-
Alkaloids	+	+	+	+
Saponins	-	-	-	-
Tannins	+	+	+	-
Steroids	+	+	+	-
Amino acids	-	-	-	-
Glycosides	+	+	+	+
Carbohydrates	-	-	-	-

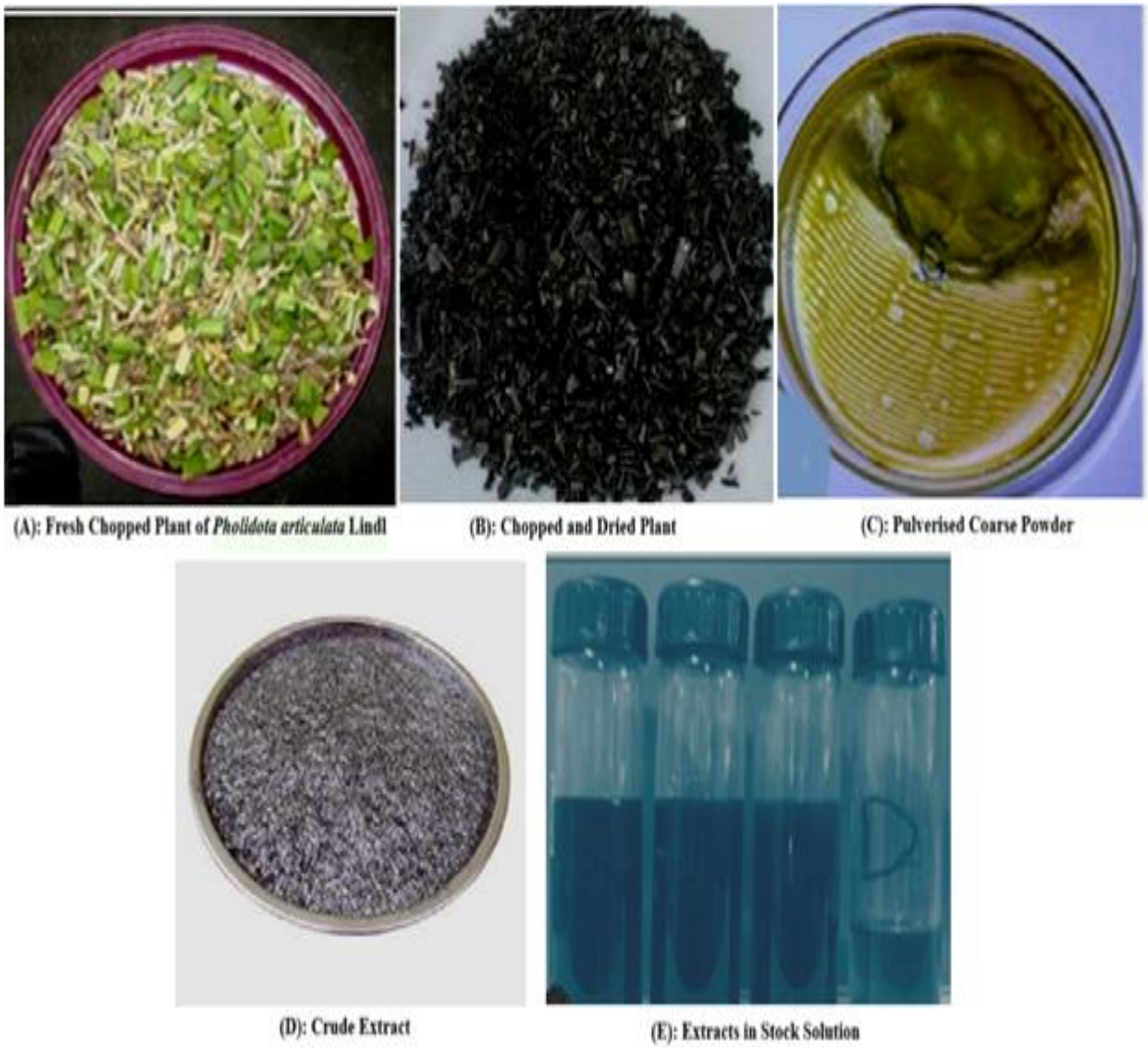

Figure 1 Extract Preparation Procedure

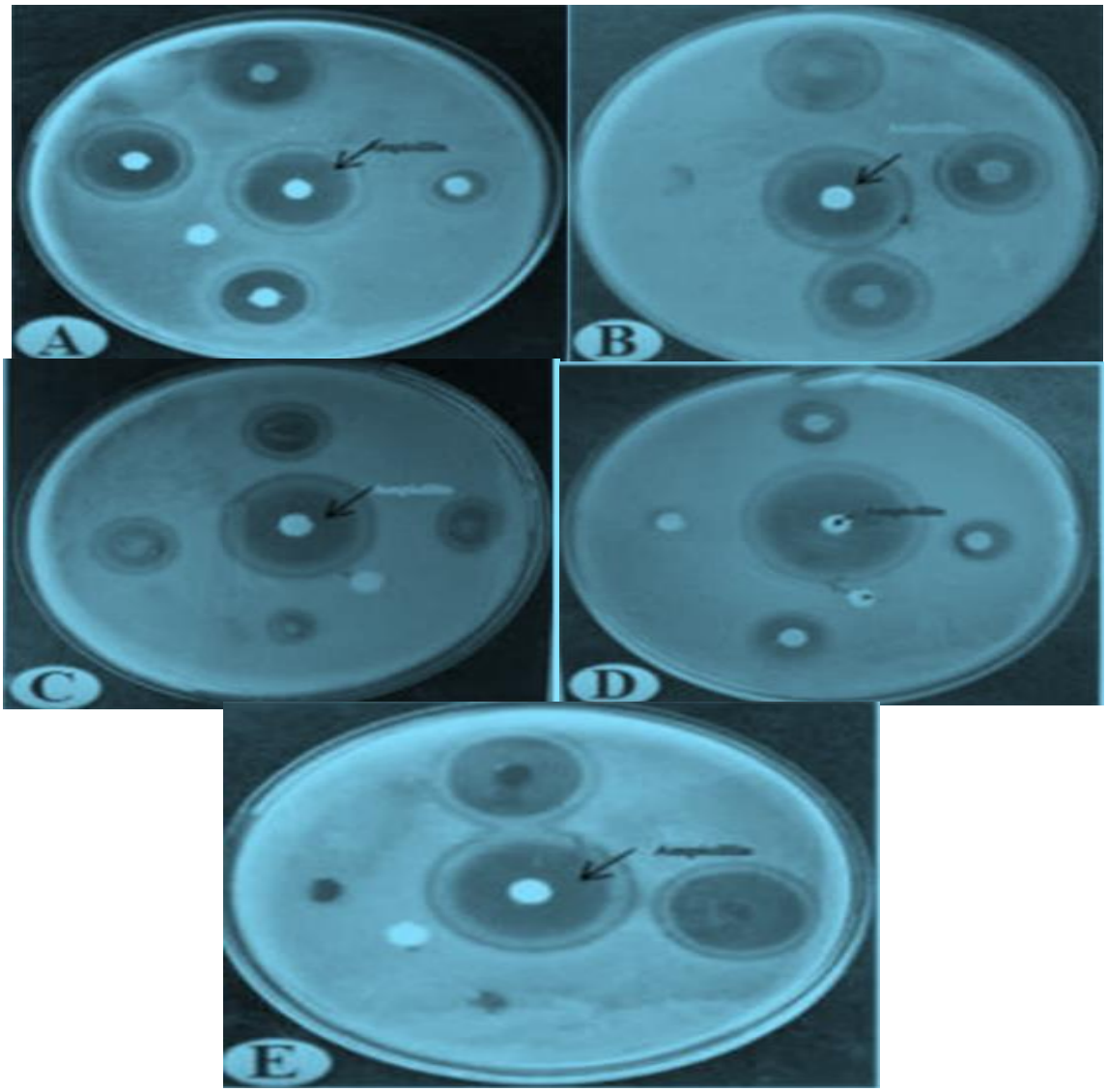

Figure 2 Antimicrobial properties of P. articulata utilising plant extract. Vibrio cholerae, Staphylococcus aureus, Klebsiella pneumoniae, Bacillus subtilis, and Escherichia coli

molecules. Human health has greatly benefited from the use of plant-derived medicines. Possible new chemotherapy drugs are created mainly based on medicinal plants, with the in vitro antibacterial test as the basis.

CONCLUSION

The findings demonstrated the strong antibacterial and antifungal qualities of several extracts from those epiphytic orchids. This finding indicates that P. articulata chloroform extracts had the highest sensitivity to preventing the growth of several bacterial and fungal species. Some phytochemicals discovered in the plant extract may be responsible for the extract's ability to control the growth of bacteria. Because of its broadspectrum antibacterial activity, P. articulata is a possibility for bio-prospecting for antibiotic and antifungal drugs. According to the study, using plants traditionally to treat diseases is appropriate. Researchers will benefit from this technique and protocol as they continue exploring the safe, powerful, and natural sources of antimicrobial and phytochemical ingredients in orchids and other plants.

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