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PHYTOCHEMICAL SCREENING AND BIOACTIVITY OF *Zingiber officinale* TO COMBAT THE MULTIDRUG-RESISTANT BACTERIAL PATHOGENS USING FOLDSCOPE

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Foldscope, is a paper-based, portable microscope with a magnification power of 140X. *Zingiber officinale* is widely used as spice, flavor and therapeutic plant in folk and conventional medicines. It is used in the regular diet in many asian countries. Chemical analysis of ginger shows that it consists of more than 400 different compounds.

Aim: In the present study, to isolate and identify the skin infection causing multidrug-resistant bacterial pathogens from clinical specimens using Foldscope (a paper microscope). To evaluate the phytochemicals and antimicrobial activity of aqueous extract of *Zingiber officinale* rhizome against the isolated MDR bacterial pathogens.

Materials and Methods: The isolates were examined by Foldscope microscopy and biochemical tests. Phyto-chemical study analyzed the existence of alkaloids, flavonoids, phenols, saponins, terpenoids, steroids, tannins, reducing sugars, carbohydrates, and amino acids. Chloramphenicol, Penicillin, Streptomycin, Methicillin, and Vancomycin, are the antibiotic disc used in the disc diffusion assay.

Results: The pathogens were isolated using Foldscope. Phytochemical study revealed the presence of flavonoids, tannins, carbohydrates and reducing sugars, saponins, Glycosides, terpenoids, and steroids. Antimicrobial activity was performed by Kirby-Bauer's–agar well diffusion method using aqueous extract of *Z. officinale* against the pathogens from wound and multidrug-resistant pattern was observed in the isolates. The crude aqueous extract of *Z. officinale* rhizome was found to be active against multidrug-resistant *Staphylococcus aureus* (20 mm), *Escherichia coli* (19 mm), *Pseudomonas aeruginosa* (22 mm) and *Klebsiella pneumonia* (19 mm). The highest zone of clearance was found in *Pseudomonas aeruginosa* (22 mm), followed by *Staphylococcus aureus* (20 mm), *Escherichia coli* (19 mm), and *Klebsiella pneumoniae* (19 mm).

Conclusion: The end result attained that the aqueous extracts have a potential resource of useful pharmaceutical bioactive and antibacterial property and it is used as a natural drug by means of itself to treat the microbial infection.

Keywords: Phytochemicals; antimicrobial; wound pathogens; multidrug-resistance.

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1. INTRODUCTION

Foldscope is the low-cost paper microscope whichever is durable, portable with magnification of 140X and 2-micron resolution. Paper microscope is invented by Manu Prakash and Jim Cybulski by Prakash lab at Stanford University, USA in 2014. The Foldscope can be used to study and understand the bacteria of clinical sample [1,2]. After that, (DBT Foldscope project), Department of Biotechnology Govt. of India, New Delhi and Stanford University Prakash Lab, USA prior signed an harmony to fetch the Foldscope (a paper microscope) to India to support interest in science discipline [3]. Pathogenicity of microbes and additional infectious diseases encompass restricted through the use of commercially existing antimicrobial drugs meant for the last several years. Incredible use of drugs has urbanized multiple drug resistance (MDR) in numerous pathogens of bacteria. As of the incredibly antique instance plough at the moment natural plants have been the foundation of lots of conventional drug systems all through the world and sustained to provide humankind by novel remedies. An enormous range of therapeutic plants, their purified bioactive and their products as of the therapeutic plants endow with infinite impunities intended for novel drug development because of the incomparable ease of access of diverse chemical compounds [4]. They are habitual of inexpensive having less adverse effects and improved efficiency in MDR outbreaks [5]. India is solitary of the nation that comprehensively makes use of herbal drugs to meet the requirements of healthcare and it is used to change the commercial antibiotics. Phytodrugs reveal their remedial abilities via an assortment of bioactive components such as alkaloids, carbohydrates, saponins, flavonoids, glycosides, gums, terpenoids, phenolic compounds, steroids, volatile oils, etc. derived from abundant species of therapeutic plants [6]. Diverse parts of these therapeutic plants are conventionally used as an ayurvedic drug in diverse regions [7,8,9]. In conventional medicine the rhizome of ginger, which is in favor of therapeutic of an extensive choice of disorder. In Folk Ayurveda, for the remedy of childish colic *Z. officinale* with water or milk in the appearance of paste are used externally. The stem of zinger (rhizome) is used while both a spice and a drug and it can be used fresh, dehydrated and pulverized, or oil or as a squash. *Z. officinale* belongs to the family Zingiberaceae, is extensively cultivated meant for its traditional therapeutic uses like cold, head pain, rheumatic and muscle disorders in and also used as a mustard and ketchup flavor [10]. Several studies encompassed the composition of phytobioactives of ginger rhizomes, gingerol, shogaol zingiberene, and their cognates as the chief components [11]. Current research has made known abundant therapeutic actions of ginger rhizome comprise the pharmacological actions of antioxidant effects, antimicrobial activity, and anti-inflammatory components [12,13,14]. In the current research, to isolate and identify the skin infection causing multidrug-resistant bacterial pathogens from clinical specimens using Foldscope (a paper microscope) and to assess the phytochemicals and antimicrobial action of aqueous extract of *Z. officinale* rhizome against the isolated MDR bacterial pathogens.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The clean, healthy, *Z. officinale* rhizome was collected as of the marketplace of Rasipuram, Namakkal District, Tamilnadu, India. The taxonomic identities of the *Z. officinale* rhizome were confirmed.

2.2 Rhizome Extract Preparation

The rhizome was exactly washed, dried under shadow, milled and stored in air-tight bottles at 4°C. Rhizome aqueous extract was ready by soaking of 50 gm powder in 250 ml water, extracted for 8 hours in the soxhlet apparatus. The temperature was maintained 95-100°C for aqueous extract. The aqueous extract was collected after 8 hours, rigorous and stored at 4°C in a sealed airtight container intended for further use [15].

2.3 Phytochemical Analysis

The intense aqueous ginger rhizome extract subjected to phytochemical analysis for screening antimicrobial actions. Phytoanalysis techniques were used for the analysis of alkaloids, flavonoids, phenols, saponins, terpenoids, tannins, reducing sugars, carbohydrates, steroids and amino acids in the extract [16,17,18,19].

2.3.1 Test for alkaloids

Mayer's test: In 60 ml and 10 ml of distilled water, 1.36 gm of $Hgcl_2$, 5 gm of KI were disbanded respectively. The aforementioned solvents were mixed watery to l00 ml using refined water. Scarce droplets of reagent were added to one ml of rhizome extract and it gives the precipitate in colorless indicates the reality of alkaloids.

Wagner's test: In 3 ml of rhizome extract, 1 ml of wagner's reagent was added and the initiation of a reddish-brown precipitate indicates the occurrence of alkaloids [20].

2.3.2 Test for flavonoids

Few droplets of watered-down HCL and minute amount of magnesium were supplemented to 0.5 ml of rhizome extract and simmered for a few minutes. The development of deep red colour showed the occurrence of flavonoids.

2.3.3 Test for saponins

5 ml of refined water was taken in a test tube and added with 0.2 g of rhizome extract and dazed robustly the blend was set aside for 3 min. The creation of a honeycomb akin to froth indicates the continuation of saponins [21].

2.3.4 Test for phenols

In the $Fecl_3$ test, sample 1ml was supplemented with refined water 2 ml, afterward scarce drops of 10% aqueous $Fecl_3$ solution. Blue or green precipitate is confirmed as the subsistence of phenols.

2.3.5 Test for tannins

Lead acetate test: Rhizome extract 5 ml was added in a test tube and supplemented with scarce droplets of 1% lead acetate. Yellow or red precipitate formation indicates the reality of tannins.

$FeCl_3$ test: An assortment of Rhizome extract 2 ml and $FeCI_3$ 2 ml for the creation of precipitate in blue or black identify the existence of tannins [22].

2.3.6 Test for steroids

Salkowski's test: In the blend of chloroform and filtered rhizome extract 5 drops of conc. sulphuric acid was added. Shaken gently and allowed to position cautiously. Golden yellow color appearance showed the presence of triterpenes (phytosterol).

1 mg of Rhizome extract was added with 2 ml of chloroform and 1 ml of conc. H_2SO_4. The formation of reddish-brown colour indicates the occurrence of steroids.

2.3.7 Test for reducing sugars

Fehling's test: Fehling's A (1 ml) and B (1 ml) solutions were an assortment of and simmered. 1 ml of Rhizome extracts were added with above said Fehling's solution and set aside for ten minutes in a boiling water bath. Yellow precipitation followed by brick red showed the subsistence of reducing sugars.

Benedict's test: 2 ml of the extract was taken in a test tube and 2 ml of Benedict's solution was varied and simmered for ten minutes in boiling water bath until the yellow, green and red colour changes confirmed the incidence of reducing sugars.

2.3.8 Test for carbohydrates

In Molisch's test, Rhizome extract is supplemented with 5 ml of refined water, 2 drops of alcoholic ά-naphthol solution and by a dropper vigilantly dispense alongside the test tube dropwise conc H_2SO_4. Violet colour formation at the combination of two liquids showed the subsistence of carbohydrates [23].

2.3.9 Test for aminoacids

In a test tube, 3 ml rhizome extract is supplemented with 3 drops of lead acetate solution (5%), boiled in a water bath for 10 mins. The purple or blue colour change the subsistence of amino acids as in the ninhydrin test [24].

2.4 Clinical Sample Collection and Culture Preparation

Multi-drug resistant bacteria secluded from thirty-six wound samples collected in germ-free containers from government hospitals at Salem and transported to the laboratory. Wound swabs inoculated in nutrient broth and subsequent to 24 hours pure culture obtained in the McConkey agar plate. The pure cultures of the isolates were maintained on nutrient agar slants.

2.5 Foldscope Microscopy

The bacterial isolates used in this study were isolated by Foldscope microscopy (a paper microscope) for their physiological identification like gram staining, motility, and negative staining.

2.6 Biochemical Analysis

Isolates were predictable according to their biochemical profilings in a similar way (utilization of citrate, indole, catalase, oxidase, carbohydrate fermentation, methyl red, Voges Proskauer, TSI and urease tests) is carried out [25].

2.7 Antimicrobial Sensitivity Test

An entirety of fifty isolates were analyzed for antibiotic sensitivity test which was carried out via disc diffusion procedure in conflict to five-grade antibiotic discs (Chloramphenicol, Penicillin, Streptomycin, Methicillin, and Vancomycin) incubated for 24 h [26]. Through a perfectly sterile swab, the chosen colonies were extended on the muller hinton agar (MHA) plate and allowed to dry up

for 2–5 min. In a while afterward, by a disc distributor, the antibiotic discs were positioned to the MHA plates and incubated at 25-30°C for 18–24 h. The zone of clearance via plate ruler was precise and expounded the isolates as Resistant (R), Intermediate (I) or Susceptible (S) [27,28].

2.8 Determination of Antibacterial Activity

The aqueous rhizome extracts were placed to attain a concentration in conflict to check organisms [29] and afterward, this inoculum was swabbed consistently onto the Muller-Hinton Agar plates and punched outwells of 6 mm diameter in all plates for antibacterial action via well diffusion protocol. Varied concentrations (20 mcg, 40 mcg, and 60 mcg) of 50 µl of aqueous extract were transferred into these wells and the inoculated plates were incubated at 37°C overnight [30,31]. The wound pathogen sensitivity pattern of aqueous extracts was deliberated the (diameter in millimeter) of the zone of inhibition (ZOI). Of late, an obvious zone of inhibition of the bacteria after (24 hr and 48 hrs) readings were observed for aqueous extract and the bacterial strain experiments were performed in thrice.

3. RESULTS

3.1 Screening of Phytochemical Analysis

The existence of flavonoids, steroids, saponins, tannins, terpenoids, reducing sugars and carbohydrates in the aqueous extract of *Z. officinale* rhizome is by means of phytochemical study as exposed in Table 1.

3.2 Foldscope Microscopy and Biochemical Characterization

The bacteria were isolated from the patient's wound samples (36nos.) and examined for Foldscope (paper microscope) and biochemical characterization (Table 2). The colony morphology of these isolates was circular, small, convex, smooth colony, large, opaque, shiny, tiny, swarming growth and translucent colonies. Beneath Foldscopic view, gram-positive and gram-negative bacterial pathogens were also observed. Biochemical scrutiny such as IMVIC, indole, catalase, oxidase, carbohydrate test, Voges Proskauer, TSI and urease tests performed and tabulated (Table 2). Among the 36 wound samples totally 50 predominant isolates were identified as *Pseudomonas aeruginosa* (15), *Staphylococcus aureus* (21), *E. coli* (8), and *Klebsiella pneumonia* (6). All the identified pathogens were characterized as catalase positive and oxidase negative.

3.3 Antibacterial Activity

Based on the antibacterial property results, the sensitive zone of inhibition for Chloramphenicol, Penicillin, and Streptomycin was observed on *Staphylococcus aureus* (18 mm to 20 mm) only, Chloramphenicol was sensitive to *E. coli* (19 mm), and the remaining antibiotics showed multidrug-resistant to the isolates. The zone of inhibition exposed by the antibiotics in opposition to the 4 isolates and the results are deliberated and tabulated (Table 3).

Noteworthy activity of aqueous extract of ginger rhizome in 60 mcg concentration showed effectual

Table 1. Qualitative detection of phytochemical constituents in *Zingiber officinale* extract

Phytochemical constituents		Aqueous extract
Alkaloids	Mayer's test	-
	Wagner's test	-
Flavonoids		+
Steroids	Salkowski's test	+
Saponins	Foam test	+
Tannins	Lead acetate test	+
	Reaction with Fec13	+
Terpenoids		+
Phenols	Ferric chloride test - Colour with Fec13	-
Carbohydrates - Reducing sugars	Molisch's test	+
	Fehling's test	+
	Benedict's test	+
Amino acids	Ninhydrin	-

+ indicates the presence of compound; '-'indicates the absence of compound

Table 2. Foldscope microscopic isolation and biochemical characterization of pathogens

Gram staining	Motility	Negative Staining	I	MR	VP	CIT	TSA	Urease	Identified pathogens
G –ve Rod	Motile	Capsule absent	**N**	N	N	P	AK/AK, G^-, H_2S^-	P	*Pseudomonas aeruginosa*
G –ve Rod	Non motile	Capsule Present	N	N	P	P	A/A, G^+, H_2S^+	P	*Klebsiella pneumoniae.*
G +ve grape like clusters of cocci	Non motile	Capsule absent	N	P	N	N	A/A, G^-, H_2S^-	N	*Staphylococcus aureus*
G –ve Rod	Motile	Capsule absent	N	P	N	N	A/A, G^+, H_2S^+	P	*E. coli*

I; Indoel, MR;Methyl Red, VP; Voges Proskauer, CIT;Citrate, TSA; Triple Sugar Iron agar; P-Postive, N-Negative, A-Acid, Ak-Alkaline, G-Gas, H_2S –Hydrogen sulfide

Table 3. Antibacterial activity of antibiotic disc against the pathogens (ZOI in mm)

Isolated pathogens	Ch 30 mcg		P 10 mcg		Str		Met		Va	
	ZOI	Inf	ZOI	Inf	ZOI	Inf	ZOI	Inf	ZOI	Inf
Pseudomonas aeruginosa	14±1	I	12±1	R	14±1	I	10±1	R	10±1	R
Klebsiella pneumoniae	10±1	R	7±1	R	6±1	R	7±1	R	7±1	R
Staphlylococcus aureus	20±1	S	18±1	S	20±1	S	8±1	R	6±1	R
E. coli	19±1	S	8±1	R	8±1	R	7±1	R	8±1	R

R–Resistance, I –Intermediate, S–Sensitivity, ZOI–Zone of Inhibition, Inf–Inference
C; Chloramphenicol, P: penicillin, Str: streptomycin, Met; Methicillin and Va; Vancomycin

Table 4. Antibacterial activity of *Z. officinale rhizome extract* against the wound pathogens

S. no	Isolated pathogens	*Zingiber officinale* aqueous extract (ZOI in mm)		
		20 mcg	40 mcg	60 mcg
1	*Pseudomonas aeruginosa*	11 mm	15 mm	22 mm
2	*Klebsiella pneumoniae*	12 mm	16 mm	19 mm
3	*Staphylococcus aureus*	11 mm	14 mm	20 mm
4	*E.coli*	9 mm	13 mm	19 mm

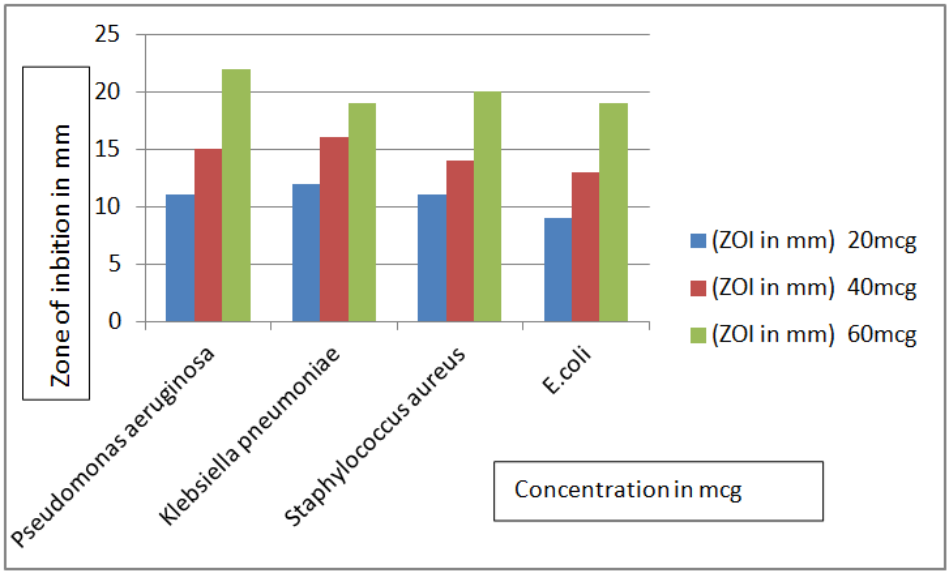

Fig. 1. Antimicrobial activity of *Z. officinale rhizome extract* against the wound pathogens

zone of inhibition in the sort of 19 mm-22 mm was observed on *Pseudomonas aeruginosa* (22 mm), followed *by Staphylococcus aureus* (20 mm), *E. coli* and *Klebsiella pneumonia* (19 mm). The zone of inhibition of *Z. officinale rhizome* extract was given in Table 4 and Fig. 1.

4. DISCUSSION

The findings of the initial phytochemical selection laid the base for additional works since it showed positive end product for flavonoids, steroids, saponins, tannins, terpenoids, reducing sugars and carbohydrates in the ginger rhizome extract. In meticulous the flavonoids were reported to exist accountable for medicinal plants [32]. Water was used while a solvent to extract the juice of *Z. officinale*. Imokawa [33] determined to facilitate the substances liable for the spicy quality of *Z. officinale* are, on the whole part, insoluble in water, while the required enzyme proteases are extracted through water. As a result, the aqueous ginger rhizome extract is non-irritable and in safe hands to use on man's skin [34]. The grades in the antimicrobial property support Jagetia et al. [35] who administered *Z. officinale* intraperitoneally and observed antimicrobial action next to *Pseudomonas aeruginosa* and *Escherichia coli*. The elevated sensitivity of *S. aureus* than that of *E. coli* to *Z. officinale* essential oil was established in [36].

5. CONCLUSION

Even though *Z. officinale* rhizome is notorious because flavor in diverse cultures and the mainstream of research have been decided on anti-vomiting and pain-reducing effects, gastrointestinal, respiratory tracts, infections with sore have to be considered. The current investigation of *Z. officinale* rhizome extract confirms that there is an elevated quantity of antibacterial actions. In conclusion, owing to the high yield of phytochemical bioactive in ginger and extensive bioeffects of ginger extract, its use in herbal formulations must be used to treat MDR pathogens and considered in numerous dissimilar sources fairly than aromatherapy.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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