Three distinct solvent extracts of Sassurea costus rhizome and leaf sections have antifungal and antibacterial properties against various infections.

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***Abstract*—In Kashmiri, Sassurea costus, also called Kuth, is used as an appetiser, digestive aid, or anti-gastric medication to treat gastrointestinal issues. The roots of it have been used to treat respiratory conditions like asthma and cough. Other traditional medical systems, such as Unani, also make use of the herb. Because of its many antifungal and antibacterial properties, Sassurea costus is utilised as a substitute for artificial preservatives that pose a health risk to people. Finding the bioactive substances in S. costus extract (SCE) and assessing its antimicrobial efficacy against a few harmful microbes were the objectives of this investigation. The results of the antimicrobial screening showed that the hexane and ethyl acetate extracts of S. costus, such as E. coli, were highly susceptible to Gram-negative bacteria among the cited microbiological strains (5 bacteria and 3 funguses). To measure the bioactive substances in SCE, GC-MS was employed.**

***Index Terms*—GC-MS, antifungal, antibacterial, Sassurea costus.**

I. INTRODUCTION

The usage of medicinal plants is a long-standing custom that has evolved along with human society. From one generation to the next, information about these plants has changed in tandem with scientific advancements, cultural conventions, and human knowledge. This gradual but steady development has led to the accumulation of a substantial body of knowledge, which is always changing as new details about the potential therapeutic benefits of natural remedies are discovered. Thus, the use of medicinal plants shows the productive intersection of traditional knowledge with state-of-the-art science [1].

For ages, people from all over the world have utilised plant-based products for medical purposes. Utilising plants for their therapeutic qualities has a long history in many cultures, and these customs have frequently been carried down through the centuries [2]. Approximately 80% of people in middle- and low-income nations rely on medicinal plants to meet their basic healthcare needs (3). Throughout human history, medicinal plants have been used for therapeutic purposes. Even today, up to 80% of the world's population, the majority of whom reside in developing nations depends upon traditional herbal medicine as their primary source of healthcare. Many of the herbal medications prescribed in conventional medical care are untested and have insufficient knowledge by scientific methods (4) Native to the subalpine regions of Jammu and Kashmir, Himachal Pradesh, and Uttarakhand, Sassurea costus (Costaceae family) is used to treat a variety of conditions, including ulcers, headaches, rheumatism, colds, coughs, and throat infections. In Korea, it is commonly used in traditional medicine to treat inflammatory conditions [5].

The roots of S. costus have high levels of flavonoids and phenolics, as well as a variety of other active phytoconstituents. Furthermore, the methanolic

extract had a considerable amount of phytochemicals [6]. In Islamic medicine, Sausurea costus, also known as S. costus, is well-known and mentioned in the Messenger of Allah's (May peace and blessings be upon him) sacred hadiths [7]. It has been utilised by conventional healers since the time of Islamic civilisation and is referred to as "Al-Kost Al-Hindi" in the Arab world. Traditionally, S. costus has been used as a sedative, bronchodilator, antiseptic, stimulant,andrepellant[8].

In Indian medical systems, Sassurea costus is used either alone or in combination with other medications. S. costus should be employed in more human-related healthcare and medicines because its roots contain antispasmodic, antifungal properties, and antibacterial qualities and are used to treat rheumatism, asthma, cough, cholera, and chronic skin conditions [9, 10, 11].

II. MATERIALS AND METHODS

Fieldwork and plant collection:

In September 2023, S. costus was gathered in the Budnambhal region of the Kupwara district in Jammu & Kashmir, India. Fieldwork was conducted at various locations within the Kupwara District Forest. In the north, west, and east, Kupwara district is steep and mountainous. The spacemen were examined in all of their various phases, and the relevant data was recoded and examinedfurther.

Identification of the specimen:

The species was determined by looking through the specimens in the herbarium and published information. The collected specimens gathered for this study were contrasted with those submitted to the Botany University of Kashmir's Centre for Biodiversity and Taxonomy the Department (vocture spacemen number 9045-KASH).

Preparing Crude Extracts

To prevent the chemical contents from decomposing, the wholesome rhizome and leaf samples of S. costus were dried in a shaded environment and then ground into a fine powdered using an electric blender. After being pulverised and extracted using a solution of Me OH, ethyl acetate, and hexane (1:1, 5 L) at room temperature for 24 hours, the 400g of leaves and 3.265g of rhizome were filtered using Whatman filter paper. Using a rotary evaporator set to 45 degrees Celsius, this process was carried out three times until it was completely dry. Antifungal and antibacterial tests were conducted using the obtained solvent extracts.

Study of plant extracts' antibacterial efficacy against microbiological strains:

Mediapreparatio:

An approximate quantity of the powder was weighed and dissolved in one litre of water that had been diluted in a cylindrical flask in order to create the nutrient agar medium. After that, cotton wool was used to plug the flasks, which were then wrapped in aluminium foil and securely sealed with masking tape. After boiling the medium to homogeneity, they were autoclave sterilised for 15 minutes at 1210 degrees Celsius. After cooling, the sterile medium was transferred into sterile petri plates and left to solidify.

Standardisedinoculumpreparation:

Using an inoculation loop, nightly organisms from an agar medium were inoculated into an appropriate nutrition broth under aseptic conditions to paper the inoculum. For 18 to 24 hours, the broth was infected at 35 degrees Celsius. The broth's density was diluted to an equivalent and kept at a comfortable temperature in the dark.

Agar-well diffusion method:

After correcting the turbidity, a sterile cotton swab was submerged in the appropriately diluted inoculate. For uniform growth, the entire surface of the Mueller-Hinton agar plate was inoculated during swabbing. Before the antimicrobial agents started working, the plates were left at ambient temperature for a few minutes to absorb any surface moisture. Applying the diffusion technique on agar wells, the antibacterial capacity and concentration of the plant extracts were examined. The plant extract concentration was 10 mg/ml, and a sterilised aluminium crock borer was used to punch wells in the agar plates. 50 µl of a plant extract concentration and 50 µl of ampicillin were employed as the negative and positive controls, respectively, in the wells. Following incubation, the antibacterial activity of the plates was evaluated and the measurement of the inhibitory zone for each extract was determined in millimetres (mm).

Antifungal action:

PDA (Potato Dextrose Agar) preparation:

In order to cultivate fungi in the lab, Potato Dextrose Agar medium was made. Petri dishes of the conventional size (100 x 15 mm) are needed for the entire experiment. PDA was prepared by mixing 39

grams of PDA powder with 1000 millilitres of water that was distilled and stirring the mixture until it was homogenised. The PDA combination was then autoclaved for 15 minutes at 121°C and 15 psi of pressure to sterilise the media. In order to allow the agar to cool down and become solid at room temperature, the culture media was then placed into petri dishes at an amount of 20 ml/dish and kept partially wrapped on the laminar airflow.

Technique for Poisoned Food Assay:

With minor modifications, the antifungal assay employing the poisoned food technique was carried out in accordance with Shrestha and Tiwari, Zaker et al., and Mohammad et al. Sterilised petri plates were filled with 2 mL of every plant concentration, followed by 20 mL of sterile melted PDA and gentle circular stirring to ensure homogenisation. With control, the identical process was carried out. Every petri dish was given time to solidify. A sterile 5 mm dimension cork borer was then used to create a 5 mm diameter disc from the progress of the 7-day-old culture. Every disc was aseptically positioned in the middle of each treatment-containing petri plate. After being taped shut, the infected petri dishes were allowed to sit at a comfortable temperature for 7days. Three duplicates of each treatment were made. A Vernier calliper was used to measure the pathogen's growth.

Data analysis tools: Using the following formula, the percentage of mycelia inhibition was determined:

$$\%\text{inhibition}=\frac{C-T}{C}\times 100$$

where T was the mycelium growth in the treatment set subtracted from the inoculum disc diameter, and C was the mycelium growth in the control group subtracted from the inoculum disc diameter.

GC-MS (gas chromatography-mass spectrometry analysis):

Was used for identification and separation Thermo Scientific's Trace GC Ultra/ISQ Singles Quadruple MS and TG-5MS fused silicon capillaries columns (30 m, 0.251 mm, 0.1 mm film thicknesses) were utilised for the GC-MS study. Helium gas was utilised as the carrier gas at a steady flow rate of 1 mL/min for GC-MS detection, and an electron ionisation apparatus with ionisation energy of 70 eV was employed. The analysis takes into account a sample injection volume of 1 μL. The temperature of the MS transfers line and injector was fixed at 280 °C. The temperature of the oven was set to start at 40 °C (hold for 3 minutes) and increase by 5 °C each minute (hold for 5 minutes) to 280 °C as the ultimate temperature. Using a percent comparative peak area, the quantification of each detected component was examined. By comparing the compounds' relative retention time as well as mass spectra with the NIST and Wiley library information of the GC-MS system, an initial determination of the compounds was carried out (12).

III. RESULTANDDISCUSSION

Antimicrobial, antiviral, and antifungal capabilities are shown by the active biological components found in many plants and herbs [13, 14, 15]. Traditional herbal medicine has been widely used in Iraq to treat a wide range of illnesses. S. costus is used as a therapeutic plant and disease-healing agent in Iraq and around the world. It has sedative, bronchodilator, repellent, stimulant, and antiseptic properties [16]. As a possible antiviral medication possibility to treat the B.1.617.2 Delta strain of SARS-CoV2, S. costus has received approval. It has bioactive substances that inhibit viral protease [17]. Furthermore, a thorough investigation of its biological activities revealed that it had anti-trypanosomal properties [18].

The complimentary inhibitor compounds found in S. costus root extract are also utilised to treat conditions like lupus erythematous systemic, rheumatoid arthritis (RA), and some respiratory disorders that are associated with the hyperactivity of complementary mechanisms [19]. According to earlier research, the root extract of S. costus has been shown to have antiviral and anti-inflammatory properties due to the presence of an inhibitory bioactive compound [20, 21]. Furthermore, S. costus extract from roots was found to exhibit a wide range of antibacterial and outstanding anticancer properties based on the actions on cell lines [22].

Morphology of S. costus Leaves and roots:

The length and width of S. costus roots measured 11–19.5 cm and 1-2.5 cm, respectively. Several morphologies were seen in the roots, including the tapering, fusiform (or conical) one that collapsed in the middle. The thin roots, on the other hand, were sturdy, light, broad, and cylindrical. They typically had ridges that ran straight or spirally, or longitudinal wrinkles with anastomose. The dried roots were horny, short, and broken; they were either brown or dull red. It

tasted bitter and had a powerful, sweet, and aromatic smell. This finding is consistent with a prior morphological investigation of S. costus roots conducted by another researcher [23], which similarly revealed that the roots were 7–15 cm long and 1.5–5 cm wide. In contrast, the membranous, irregularly serrated leaves of S. costus exhibit the following

TraitsBaseauricledthere:
Size: Higher leaves are less large and sub sessile or have short petioles, whilst base leaves are very huge, measuring 0.50–1.25 m in length.

Antibacterial:
In the current study, an inhibition zone (IZ) of 6 mm indicates that the extract has no antimicrobial activity (the hole's zone on the agar plate is 6 mm); an IZ of 6 mm to less than 10 mm indicates weak antimicrobial activity; an IZ of 10 mm to 12 mm indicates a medium antimicrobial agents activity (the doubling of the hole diameter); and an IZ of 12 mm indicates apparent or strong antimicrobial activity (24).
A well diffusion test was used to evaluate the antibacterial activity of the S. costus root and leaf extracts in vitro. The results were interpreted using the growth of bacteria within the inhibition zone's millimetre-scale diameter. Table 1 and figure 1 displays the in vitro bactericidal activity of S. costus methanol, ethyl acetate, and hexane extract against bacteria such as the bacteria S. aureus salmonella, E. coli, proteus, and cons spp. S. costus leaf extract exhibits stronger antibacterial action against E. coli. S. costus rhizome ethyl acetate exhibits strong antibacterial action against salmonella. Gram-negaitive bacteria were shown to be more vulnerable to the antibacterial activity of methanol and ethyl acetate extracts of S. costus roots.
Staphylococcus saprophytic us, ATCC 43867 (14.5±0.0, 15.5±0.5 mm), Staphylococcus epidermis's, ATCC 12228 (13.5±0.5, 15.5±0.5 mm), and Staphylococcus aureus ATCC 29213 (11.0±0.0, 11.5±0.5 mm) are the next most susceptible bacteria, followed by Bacillus cereus ATCC 10876 (16.0±0.0, 15.5±0.5 mm). Streptococcus pneumonia ATCC 49619, on the other hand, demonstrated extremely low susceptibility, measuring 6.5±0.5 mm for both methanolic and ethanolic extracts. Conversely, the Gram-negative bacteria showed little to no sensitivity. Proteus vulgaris ATCC 6380 (9.0±0.0, 9.0±0.0 mm), Escherichia coli ATCC 25922 (8.5±0.5, 8.5±0.5 mm), and the microorganism Pseudomonas ATCC 9027 (9.5±0.5, 7.5±0.5 mm) were shown to have weak susceptibility to methanolic and ethanolic extracts respectively (25)

Table1: Effect of plant extract with concentration on different test pathogens.

Plant		S .costus rhizome			S. costus leaves		
Zone of inhibition							
Test pathogen	Conc. Mg/ml	MeOH	EtOAc	hex	MeOH	EtoAc	Hex
S.aureus	10	-	-	-	-	-	-
Salmonella	10	-	16	12	17	16	18
E.coli	10	-	13	14	18	18	18
Proteus	10	-	-	-	-	-	-
Cons	10	-	-	-	-	-	-

Conc, = (concentration), Me OH= (Methanol), EtoAc = (ethyl acetate), Hex= (hexane

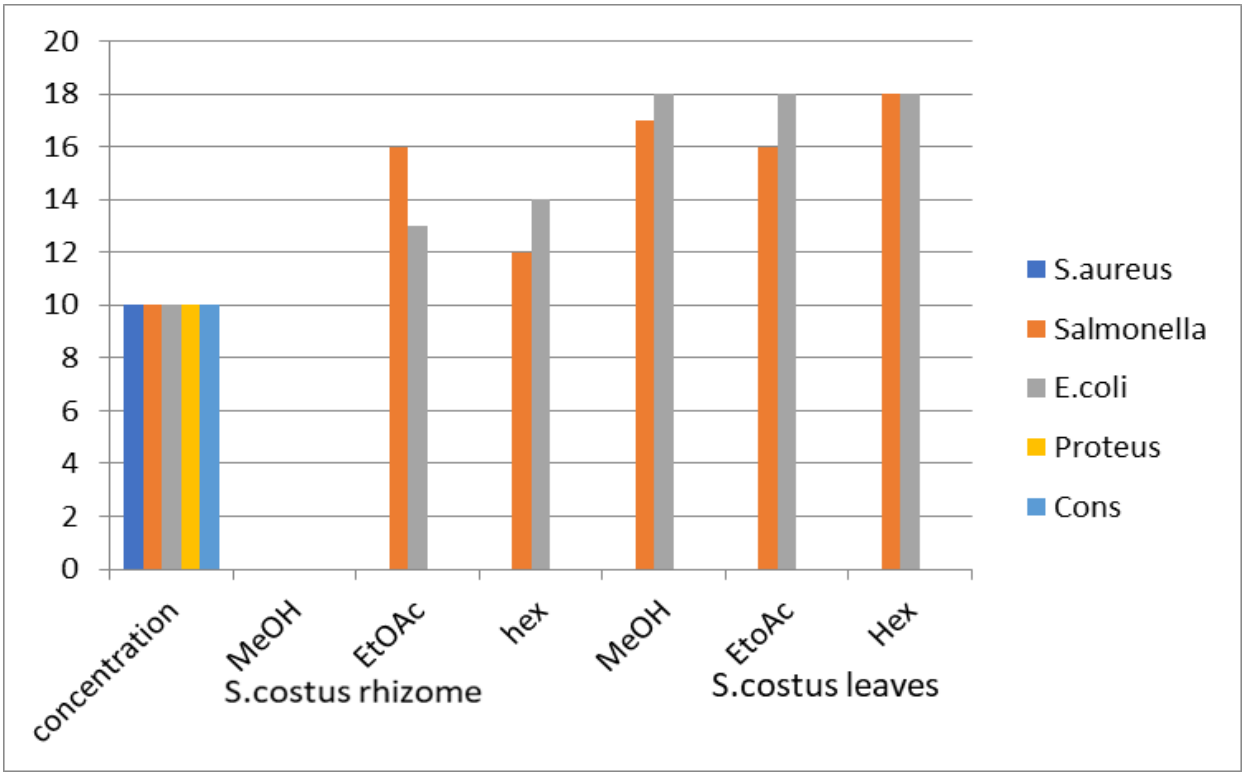

Figure 1: Comparison between inhibitory zones of methanol, Ethyl acetate and Hexane extract of S. costus against different pathogens

Antifungal activity:

The antifungal properties of S. costus of rhizome and leaves using poisoned food technique the result showed the presence of inhibitory zones diameters was used to determine the pathogen activity. As shown in table 2 figure 2 & 3 respectively.

Table2: antifungal effect of different concentrations of plant extracts on test fungal strains.

Plant		S. costus/rhizome	S. costus/leaves
%inhibition			
Fungal strain	Conc.mg/ml	EtoAc	Meoh
Alternaria Solani	100	74	62
	200	75	73
	300	82	75
Fusarium oxysporum	100	72	41
	200	76	44
	300	78	48
Collectotrichum	100	54	47
	200	61	56
	300	72	64

Conc= concentration Me OH= (Methanol), EtoAc = (ethyl acetate)

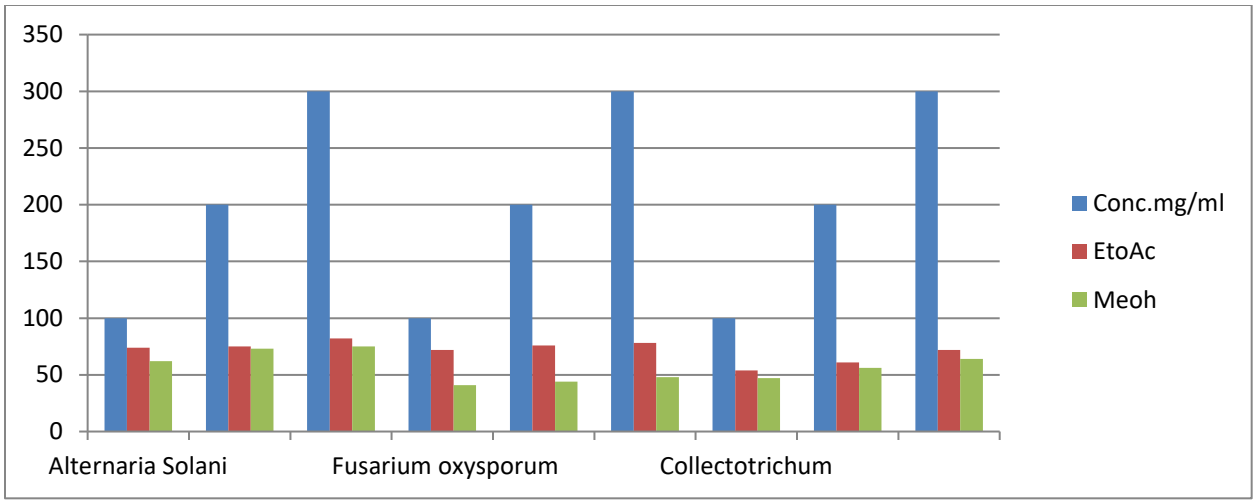

Figure: 2 Comparison between inhibitory zones of methanol, and Ethyl acetate extract of S. costus against different fungal strains.

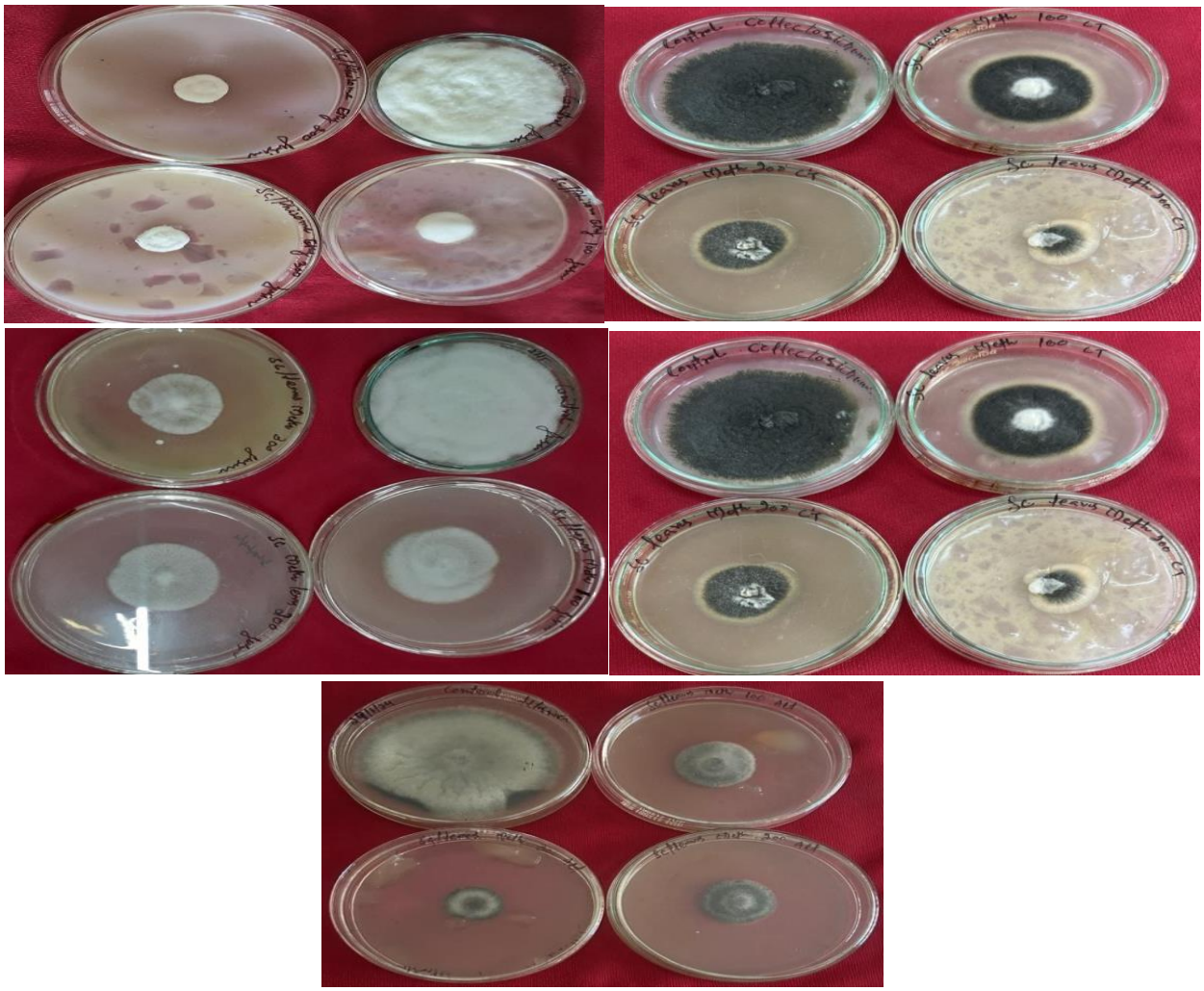

Figure 3: plant extract of roots and leaves of different concentrations showing Antifungal activity.

GC-MS study of various S. costus solvent extracts.

The chemical components found in the methanol, ethyl acetate, and hexane extracts of the Sassurea costus plant are identified using the GC/MS technique. The primary components, along with their retention durations and proportional share of the overall peak area, were displayed in the Sassurea costus GC/MS analysis results. In the methanolic ethyl acetate and hexane extracts, they resulted in the identification of 14 (Table 3), 12 (Table 4), and 9 (Table5)compounds,respectively.

Table 3: shows the peak area (percent) and retention time (RT) of the various chemicals identified in the rhizome portion of the methanolic extract of Sassurea costus that underwent GC-MS analysis.

S.no	Compounds	Molecular formula	RT	Peak area %
1	2-Azido2,4,4,6,6,8,8-heptamethylnonae	$C_{16}H_{33}N_3$	5.665	1.83
2	1-(10,10-Dimethyl-3,3dioxo-3-thia-4-azatricyclo(5.2.1.0(1,5)dec—4-yl)-3-methylpent-4-en-1-one.	$C_{16}H_{25}NO_3S$	8.230	1.68
3	Thujopsene-(12)	$C_{15}H_{24}$	10.401	1.62
4	Cyclohexanemethanol,4-ethenyl-alpha,alpha,4-trimethyl-3-(1-methylethenyl)1R-(1,alpha,3alpha,4beta)	$C_{15}H_{26}O$	11.905	6.69
5	Methyl 8,9-octadecadienoate	$C_{19}H_{34}O_2$	12.419	8.05
6	2-(2R,4aR)-4a-8-dimethyl1,2,3,4,4a,5.6,7,octahydronaphthalen2-yl)prop-2-en-1-ol	$C_{15}H_{24}O$	15.957	15.72
7	2(3H)-Benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7(-1-methylethenyl)-,(3s-3alpha,3a.alpha.,6.alpha.7.beta.,7a.beta.).	$C_{15}H_{22}O_2$	16.938	2.65
8	2(3H)-Benzofuranone,6-ethenylhexahydro-6-methyl-3-methylethenyl)-7-(1-methylethenyl) 3aS,3a.alpha.,6.alpha.7.beta.,7a.beta.).	$C_{15}H_{20}O_2$	17.991	100.00
9	Cyclodecacyclotetradecene,14,15,-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro-	$C_{22}H_{32}$	20.661	2.88
10	Azuleno(4,5-b)furan-2(3H)-one,decahydro-3,6,9-tris(methylene)-,(3aS-,3a,alpha,6a.,alpha.,9a.,alpha,9b.,beta)	$C_{15}H_{18}O_2$	21.441	6.12
11	Reynosin	$C_{15}H_{20}O_3$	24.923	10.99
12	Isolongifolol	$C_{15}H_{26}O$	27.254	1.20
13	Pregn-5-en-20-one,3-hydroxy	$C_{21}H_{32}O_2$	32.827	18.41
14	Stigma sterol	$C_{29}H_{48}O$	39.750	3.14

Table4: The retention time (RT) and peak area (percent) of the different compounds that were found in an ethyl acetate extract of Sassurea costus that were analysed by GC-MS.

S.no	Compound	Molecular Formula	RT	Peak area %
1	Cyclohexane-1,3-butadienyldene	$C_{10}H_{14}$	4.025	1.20
2	Nephthalene,decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-,(4aR-trans)	$C_{15}H_{24}$	10.441	2.21
3	Cyclohexanemethanol,4-ethenyl-3-(1-methylethenyl)-,(1R-,1.alpha.,3.alpha.,4.beta.)	$C_{15}H_{26}O$	12.017	7.71
4	7-Isopropenyl-1-4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-0ne	$C_{15}H_{22}O$	14.992	2.05

5	2-(2R,4aR,8aS)-4a-Methyl-8-methylenedechydronaphthalen-2-yl)prop-2-en-1-0l.	$C_{15}H_{24}O$	15.314	4.44
6	2(3H)-Benzofuranone,6-ethenylhexahydro-6-methyl-3-methylethenyl)-7-(1-methylethenyl) 3aS,3a.alpha.,6.alpha.7.beta.,7a.beta.).	$C_{15}H_{20}O_2$	18.466	1.42
7	Cyclodecacyclotetradecene,14,15,-didehydro-1,4,5,8,9,10,11,12,13,16,17,18,19,20-tetradecahydro-	$C_{22}H_{32}$	21.015	10.01
8	Azuleno(4,5-b)furan-2(3H)-one,decahydro-3,6,9-tris(methylene)-,(3aS-,3a,alpha,6a.,alpha.,9a.,alpha,9b.,beta)	$C_{15}H_{18}O_2$	22.084	100.00
9	Methyl 7,8-octadecadlenoate	$C_{19}H_{34}O_2$	23.660	2.21
10	Santa marine	$C_{15}H_{20}O_3$	24.504	4.29
11	Reynosin	$C_{15}H_{20}O_3$	25.059	3.75
12	Pregn-5-en-20-one,3-hydroxy	$C_{21}H_{32}0_2$	32.875	6.11

Table 5: The retention time (RT) and peak area (percent) of the different compounds that were found in a hexane extract of Sassurea costus that were analysed by GC-MS.

S.no	Compound	Molecular formula	RT	Area peak %
1	Cyclohexanemethanol,4-ethenyl-3-(1-methylethenyl)-,(1R-,1.alpha.,3.alpha.,4.beta.)	$C_{15}H_{26}O$	12.033	18.37
2	Gamma-HIMACHALENE	$C_{15}H_{24}$	13.866	4.22
3	2-(2R,4aR,8aS)-4a-Methyl-8-methylenedechydronaphthalen-2-yl)prop-2-en-1-0l.	$C_{15}H_{24}O$	15.402	12.82
4	2(3H)-Benzofuranone,6-ethenylhexahydro-3,6-dimethyl-7(-1-methylethenyl)-,(3s-3alpha,3a.alpha.,6.alpha.7.beta.,7a.beta.).	$C_{15}H_{22}O_2$	17.107	3.58
5	2(3H)-Benzofuranone,6-ethenylhexahydro-6-methyl-3-methylethenyl)-7-(1-methylethenyl) 3aS,3a.alpha.,6.alpha.7.beta.,7a.beta.).	$C_{15}H_{20}O_2$	18.418	1.99
6	Methyl 8,9-octadecadienoate	$C_{19}H_{34}O_2$	23.532	1.41
7	Aromadendrene oxide-(2)	$C_{15}H_{24}0$	24.384	1.31
8	Reynosin	$C_{15}H_{20}O_3$	24.899	1.11
9	Pregn-5-en-20-one,3-hyrlrnxy	$C_{21}H_{32}O_2$	32.803	3.08

IV. CONCLUSION

Sassurea costus (S. costus) roots are used extensively in traditional medicine; they are often cited in Islamic medicine, old Indian medicine, and Chinese medicine. Strong active ingredients found in SCE have the potential to be effective antimicrobial and antifungal substances against a variety of pathogens. These findings may pave the way for their application as a substitute for conventional chemical preservatives in food, which pose a health risk to people. According to this study, S. costus extracts can be used to treat some diseases in place of antibiotics and can be quite effective in the defence against human multi-resistant bacteria.

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