



Faculty of Medical and Health Sciences, University of Poonch Rawalakot

Journal of Pharma and Biomedics

ISSN: 3007-1984(online), 3007-1976 (Print)

<https://www.jpbsci.com/index.php/jpbs>


Antimicrobial Activity of Different Plant Extracts and Marine Algae Found in Pakistan against Clinically Important Bacteria and Fungi

Maham Jameel¹, Aisha Zafar¹, Sadiyah Saleem², Kanwal Ibrahim¹, Ghulam Hussain Jatoti³, Sadia Khalil¹¹ Department of Microbiology, Federal Urdu University of Arts Science & Technology, Karachi, Pakistan.² Department of Microbiology, University of Karachi, Karachi, Pakistan.³ Department of Plant Pathology, Sindh Agriculture University, Tandojam, Sindh, Pakistan.

Received: December 22, 2025;

Revised: March 27, 2026;

Accepted: March 30, 2026

ABSTRACT

The escalating problem of antimicrobial resistance has fueled the investigation into plant and marine source-based bioactive molecules as novel antimicrobials. This research was conducted on the antimicrobial potential of methanol, ethanol, chloroform, diethyl ether, and aqueous extracts of leaves of *Carica papaya*, leaves of *Annona squamosa*, and flowers of *Bauhinia variegata* (three indigenous plants found in Pakistan) and green algae *Codium fragile* from Hawksbay Beach in Karachi. The antimicrobial effect was tested against twelve bacterial and nine fungal species using the agar disc diffusion technique. Minimum inhibitory concentration was estimated via series of twofold dilutions in DMSO. Bacterial extracts of *B. variegata* flowers showed the widest spectrum of inhibition for different solvents (from 7 mm to 18 mm inhibition zone), with a significant activity observed against MRSA (methanol; 17 mm) and XDR-*S. typhi*. *C. fragile* showed significant inhibition against sensitive and drug-resistant strains, especially XDR-*S. typhi* (ethanol; 15 mm) and *Staphylococcus epidermidis* (diethyl ether; 16 mm). Testing with fungi has shown that Custard apple and Orchid (*B. variegata*) have selective antifungal properties for *Candida kursii* and *Candida lusitanae*. It is significant to note that none of the extracts exhibited inhibitory properties when dissolved in distilled water, indicating that solvent polarity is a crucial factor in the extraction of bioactive molecules. All active extracts had their MICs recorded at 0.8 CFU/mL. This information provides ample proof for the medicinal importance of plants and algae in Pakistan.

Keywords: Antimicrobial resistance; *Carica papaya*; *Annona squamosa*; *Bauhinia variegata*; *Codium fragile*; Disk diffusion; MRSA; XDR; *Salmonella typhi*; Antifungal; Pakistan.

Corresponding Author: Sadia Khalil

Email: sadia.khalil@fuuast.edu.pk

INTRODUCTION

The history of the interaction between humans and plants used for medicinal purposes dates back many thousands of years ago when modern synthetic drugs had not yet emerged, plants played a crucial role in healing diseases known at that time. Before 500 B.C., the use of plants was not limited to their physiological functions; people also considered them to be spiritually significant objects possessing healing power beyond any normality. By the time 14th century came, plant knowledge became

widespread in various parts of the world; the Chinese, Indians, Muslims, and Europeans traded plant substances, including such ingredients as ginger, cardamom, cinnamon, and sage, which they utilized against illnesses such as smallpox and syphilis (Fabricant & Farnsworth, 2001).

In modern times, even with the prevalence of synthetics, plants continue to provide the main source of primary healthcare. According to WHO, as much as 70% of the world's population still depends on plant-derived drugs for their essential healthcare, and up to 35,000 to 70,000 species

of medicinal plants have already been identified, constituting up to 28% of all plant species on earth (WHO, 2019). Currently, there are more than 50 key pharmaceutical drugs used clinically whose development is based on natural plant compounds, but from the roughly 250,000 higher plant species existing, only about 17% have been thoroughly tested scientifically (Newman & Cragg, 2020). These facts illustrate the immense and largely underexploited biological diversity contained within medicinal plants a diversity that scientists are now looking towards amid the looming health crisis. The rising phenomenon of antimicrobial resistance (AMR) is becoming one of the key health concerns of the current era. The excessive and inappropriate utilization of traditional antibiotics has resulted in the emergence of multidrug-resistant (MDRs) and extensively drug-resistant (XDRs), reducing the effectiveness of all the known classes of antibiotics. Examples of such organisms include methicillin-resistant *Staphylococcus aureus* (MRSA) and XDR *Salmonella typhi*, the latter is especially a grave / serious threat in Pakistan, where XDR typhoid outbreaks have been reported since 2016 (Klemm et al., 2018; WHO, 2019). Within the context of such circumstances, plant-based antimicrobial drugs present themselves as an effective solution, providing diverse compounds that can target more than one molecular structure at the same time while also having a proven safety record owing to their prolonged usage over many years (Gyawali & Ibrahim, 2014). Marine algae, in this regard are also increasingly being studied for their ability to generate new bioactive compounds. The macroalgae, that comprise green, red, and brown algal phyla, are recognized as producers of a wide array of secondary metabolites comprising polysaccharides, polyphenols, lectins, and halogenated compounds, with many of these compounds showing strong antibacterial and antifungal activities (Pereira, 2018). *Codium fragile* is an example of a green macroalgae that goes by the name of 'dead man's fingers' and that occurs along the coastlines of Sindh and Baluchistan provinces of Pakistan. This particular marine alga has been previously studied as an antimicrobial agent but with no extensive testing conducted on clinically resistant strains in Pakistan (Khalil et al., 2020).

The flora of Pakistan as well as its coastal life form a very interesting subject matter for such study. Due to its varied geographical features like subtropical plains, high altitude forests, and long coastlines along the Indian Ocean, Pakistan houses many types of plants and algae that are used ethnically for medicinal purposes. Most importantly, there is no documentation of their effects on various infections, which makes them suitable to be studied further in

laboratory environments. Apart from that, the availability and cost effectiveness of plant materials also contribute to making Pakistan a perfect destination for bioengineering studies (Ahmed et al., 2013).

The current study aimed to bridge this research gap through determination of antimicrobial activity of five different solvent extract of *Carica papaya* leaves, *Annona squamosa* leaves and *Bauhinia variegata* flowers (collectively known as papaya, custard apple and orchid or camel's foot tree, respectively), along with green algae *Codium fragile*, against twelve bacterial and nine fungal species, including drug resistant ones such as MRSA and XDR *S. typhi*.

MATERIALS AND METHODS

Materials and Sample Collection Sites

The following four biological samples were chosen due to their recognized ethnomedicinal importance and local availability:

Carica papaya (L.): Leaf samples were gathered from various domestic and agricultural habitats in Karachi, Sindh Province. *Carica papaya* is one of the most commonly grown plants in Sindh and Punjab and has a rich tradition of being used in traditional remedies against fever, inflammation, and microbes (Zunjar et al., 2016).

Annona squamosa (L.): Also known as Custard Apple or Sugar Apple, leaf samples were collected from orchards in Karachi, Sindh. The plant hails originally from tropical America but is well-established in the Sindh Province of Pakistan and has had many medicinal applications including skin diseases, toothache, and diarrhea (Jamkhande et al., 2014).

Bauhinia variegata (L.): Also known as Kachnar or Orchid tree, flower samples were gathered from the subtropical mountains of Punjab, Pakistan. There is an extensive literature on the antimicrobial, anti-inflammatory, and antidiabetic effects of different species of *Bauhinia* genus (Kuo et al., 2002). *Codium fragile* (*Suringar*) *Harriot*: The specimens of this green macroalga species were collected from Hawksbay Beach, Karachi. Hawksbay Beach is located on the coastline of the Arabian Sea and has a somewhat diverse algal intertidal zone. *Codium fragile* is characterized by a dark green color, spongy structure, and dichotomous branches. It is also locally known by different names like dead man's fingers and green sea fingers (Oh et al., 2010).

Preparation and Extraction of Samples

All samples of plants and algae collected were carefully cleaned using tap water, after which distilled water was used to perform a final washing to get rid of the contaminants. Thereafter, the samples were dried by spreading them out in absorbent papers to air-dry for seven to fourteen days away

from sunlight. After drying, the materials were pulverized into fine powders by using an electric grinder. The powdered samples were then stored in tightly capped glass bottles before extraction procedures.

The method of extraction used in this experiment is the maceration technique, since it is inexpensive and simple to use. It was chosen due to its suitability in extracting

thermolabile bioactive compounds. A 50-gram portion of each sample, in the form of powders, was introduced into a glass conical flask, with a lid, and immersed in five different solvents, namely methanol, ethanol, chloroform, diethyl ether, and distilled water. Extraction was allowed to take place at room temperature for seventy-two hours with occasional shaking at intervals of eight to twelve hours.



Figure 1: Ground powder of all four study specimens before maceration: Algae (*Codium fragile*), Custard Apple (*Annona squamosa*), Orchid/Kachnar (*Bauhinia variegata*), and Papaya (*Carica papaya*).



Figure 2: Fresh specimens of *Codium fragile* ready for air drying and further processing collected from Hawksbay Beach, Karachi.



Figure 3: Dried specimens of study organisms kept before grinding and maceration.

After maceration, the resulting solution was then filtered using Whatman No. 1 filter paper. Rotary evaporation was

conducted to strip off the solvents by applying reduced pressure at 40°C. Dimethyl sulfoxide (DMSO) was used to

dissolve concentrated extracts for testing purposes. This solvent has virtually no effect on microorganisms at such

low concentrations and is commonly utilized as a dissolving agent for plant extract (Cos et al., 2006).



Figure 4: Rotary evaporator used in removing solvents and concentrating extracts.



Figure 5: Filtration process involved in extraction where the marc is separated from the liquid extract.

Bacterial and Fungal Strains

A total of twelve bacterial and nine fungal strains have been employed for this study. The bacterial strains included: *Salmonella typhi* (Sensitive strain); XD-R *Salmonella typhi*; *Staphylococcus aureus*; Methicillin Resistant *Staphylococcus aureus* (MRSA); *Staphylococcus epidermidis*; *Pseudomonas aeruginosa*; *Klebsiella pneumoniae*; *Escherichia coli*; *Proteus mirabilis*; *Proteus vulgaris*; *Vibrio cholerae*; and *Streptococcus pyogenes*. All bacterial cultures were kept on nutrient agar slants in the Department of Microbiology, FUUAST, Karachi. The fungal strains included: *Aspergillus flavus*; *Aspergillus niger*; *Aspergillus fumigatus*; *Trichophyton mentagrophytes*; *Rhizopus sp.*; *Mucor sp.*; *Candida albicans*; *Candida tropicalis*; *Candida glabrata*; *Candida kursii*; and *Candida lusitanae*.

Diffusion Assay

Antibacterial and antifungal activities were carried out by

the agar disk diffusion test based on CLSI guidelines (CLSI, 2021). The inoculums for each bacterial species were prepared using sterile normal saline with their turbidities adjusted to a 0.5 McFarland standard, which is equivalent to approximately 1×10^8 CFU/mL.

For bacterial testing, nutrient agar medium and for fungal testing, Sabouraud dextrose agar medium were prepared and sterilized at 121°C for 15 minutes before being dispensed in 25 mL portions into sterile 90 mm petri plates. Once solidified, the surface of each plate was inoculated by evenly spreading a prepared bacterial or fungal suspension using sterile cotton swabs. Sterile filter paper discs of 6 mm diameter impregnated with 20 μ L each of the different plant or algal extract were placed onto each plate. Negative control was made by loading DMSO into the discs. Plates were incubated at 37°C for 24 hours and at 25°C for 48 hours. After incubation, the size of the inhibition zones produced around the filter paper discs was measured using a

Vernier caliper in mm. Zones were considered positive if they showed clear and defined inhibition zones larger than the disc sizes (> 6 mm).

Determination of Minimum Inhibitory Concentration (MIC)

The extracts showing promising results in terms of antibacterial or antifungal properties by disk diffusion test, were further tested for the minimum inhibitory concentration preparing two-fold serial dilutions of the extracts in DMSO such that the final concentration was either 0.8, 0.4, or 0.2 CFU/mL. The diluted extracts were then tested in the same manner as described previously.

RESULTS

Antibacterial Activity of *Codium fragile* (Marine Algae) Extracts

Among all organisms tested, extracts of *C. fragile* displayed inhibition against eight bacterial species, with aqueous extracts (distilled water) showing no antibacterial effects on any organisms. The most significant inhibition effects were shown against *S. epidermidis* using diethyl ether (16 mm), and against XDR-*S. typhi* using ethanol (15 mm). Consistent inhibition of *Pseudomonas aeruginosa* was noted for all four organic solvents (10–13 mm).

Table 1: Antibacterial efficacy of *Codium fragile* extracts towards twelve bacterial species; inhibition zone in mm; (–) = inactive.

Bacteria	Met	Eth	Chl	Die	LE	FU	AZ	CEF	CE	DW
<i>Salmonella typhi</i>	10	10	11	13	31	17	11	34	32	–
XDR- <i>S. typhi</i>	11	15	10	08	-	-	-	08	09	–
<i>S. aureus</i>	11	12	–	–	37	28	-	28	28	–
MRSA	–	12	–	12	-	-	-	-	-	–
<i>S. epidermidis</i>	–	11	–	16	34	-	-	-	-	–
<i>P. aeruginosa</i>	13	12	10	10	26	19	18	14	13	–
<i>K. pneumoniae</i>	–	10	–	08	30	7	-	36	28	–
<i>E. coli</i>	–	–	–	10	40	19	17	39	23	–
<i>Proteus mirabilis</i>	–	–	–	–	39	18	11	37	45	–
<i>Proteus vulgaris</i>	–	–	–	–	39	16	13	40	46	–
<i>Vibrio cholerae</i>	–	–	–	–	25	28	20	21	23	–
<i>S. pyogenes</i>	–	–	–	–	27	13	-	24	19	–

(Met): Methanol extract, (Eth): Ethanol extract, (Chl): Chloroform extract, (Die): Diethyl ether, (LE): Levofloxacin, (FU): Fusidic acid, (AZ): Azithromycin dihydrate, (CEF): Cefotaxime, Sodium, (CE): Ceftriaxone sodium

MRSA (methicillin-resistant *S. aureus*), known for its resistance to drugs, was sensitive to the extracts from *C. fragile* in ethanol (12 mm) and diethyl ether (12 mm). No antibacterial effects were observed against *P. mirabilis*, *P. vulgaris*, *V. cholerae*, and *S. pyogenes*.

Antibacterial Action of *Annona squamosa* (Custard Apple Leaves) Extracts

The custard apple leaf extracts exhibited antibacterial effects against seven different bacteria, with the strongest effects found in the ethanol and diethyl ether extracts. It is

interesting that methanol and ethanol extracts exerted antibacterial effects on MRSA, measuring 10 mm and 15 mm, respectively. XDR *S. typhi* showed antibacterial effects solely in the presence of diethyl ether extract (11 mm). The bacteria *Staphylococcus aureus* showed antibacterial effects in the ethanol extract (9 mm). *E. coli* exhibited antibacterial effects in methanol (data not shown), ethanol, chloroform, and diethyl ether extracts. Distilled water extracts exerted no antibacterial effect on the tested bacteria.

Table 2: Zone of inhibition for antibacterial action by extracts from *Annona squamosa* leaves (custard apple); results shown in mm; (+) = presence of activity but zone not accurately measurable; (–) = lack of activity.

Bacteria	Met	Eth	Chlo	Die	LE	FU	AZ	CEF	CE	DW
<i>S. typhi</i>	–	10	08	07	31	17	11	34	32	-
XDR- <i>S. typhi</i>	–	–	–	11	-	-	-	08	09	-
<i>S. aureus</i>	–	09	–	–	37	28	-	28	28	-

MRSA	10	15	–	–	–	–	–	–	–	–
<i>S. epidermidis</i>	12	12	–	15	34	–	–	–	–	–
<i>P. aeruginosa</i>	12	12	–	08	26	19	18	14	13	–
<i>K. pneumoniae</i>	–	–	07	07	30	7	–	36	28	–
<i>E. coli</i>	+	+	+	+	40	19	17	39	23	–
<i>Proteus mirabilis</i>	–	–	–	–	39	18	11	37	45	–
<i>Proteus vulgaris</i>	–	–	–	–	39	16	13	40	46	–
<i>Vibrio cholerae</i>	–	–	–	–	25	28	20	21	23	–
<i>S. pyogenes</i>	–	–	–	–	27	13	–	24	19	–

(Met): Methanol extract, (Eth): Ethanol extract, (Chl): Chloroform extract, (Die): Diethyl ether, (LE): Levofloxacin, (FU): Fusidic acid, (AZ): Azithromycin dihydrate, (CEF): Cefotaxime, Sodium, (CE): Ceftriaxone sodium

Antibacterial Action of Bauhinia variegata (Camel's Foot Tree Flowers) Extracts

Flower extracts from *Bauhinia variegata* were found to be the most potent and consistent antibacterial substances among all the tested samples. Positive inhibition of bacteria growth was recorded for nine out of twelve species tested using all four organic solvent types. The largest zones of inhibition were obtained against *Pseudomonas aeruginosa*

in all solvents used; 18 mm in diethyl ether and 15 mm in methanol. There was strong inhibition of MRSA observed in methanol (17 mm) and chloroform (13 mm) as well. XDR-*S. typhi* was inhibited by three solvents; methanol (12 mm), ethanol (14 mm), and diethyl ether (12 mm). *E. coli* bacteria resistant to other extracts were effectively inhibited by all solvents (8-10 mm). *S. aureus* was inhibited by all solvents (10-15 mm).

Table 3: Antibacterial properties of the flower extracts from *Bauhinia variegata* (camel's foot tree); zone of inhibition in mm; (–) = negative.

Bacteria	Met	Eth	Chlo	Dieth	LE	FU	AZ	CEF	CE	DW
<i>S. typhi</i>	10	–	–	–	31	17	11	34	32	–
XDR- <i>S. typhi</i>	12	14	–	12	–	–	–	08	09	–
<i>S. aureus</i>	12	12	10	15	37	28	–	28	28	–
MRSA	17	–	13	10	–	–	–	–	–	–
<i>S. epidermidis</i>	15	13	11	15	34	–	–	–	–	–
<i>P. aeruginosa</i>	15	12	14	18	26	19	18	14	13	–
<i>K. pneumoniae</i>	10	07	–	08	30	7	–	36	28	–
<i>E. coli</i>	08	10	10	08	40	19	17	39	23	–
<i>Proteus mirabilis</i>	–	–	–	–	39	18	11	37	45	–
<i>Proteus vulgaris</i>	–	–	–	–	39	16	13	40	46	–
<i>Vibrio cholerae</i>	–	–	–	–	25	28	20	21	23	–
<i>S. pyogenes</i>	–	–	–	–	27	13	–	24	19	–

(Met): Methanol extract, (Eth): Ethanol extract, (Chl): Chloroform extract, (Die): Diethyl ether, (LE): Levofloxacin, (FU): Fusidic acid, (AZ): Azithromycin dihydrate, (CEF): Cefotaxime, Sodium, (CE): Ceftriaxone sodium

Antibacterial Properties of Extracts from Carica papaya (Papaya Leaf)

Extracts from papaya leaves demonstrated their antibacterial properties against seven types of bacteria, but the largest zones were noted for *S. aureus* in methanol (22 mm) and diethyl ether (19 mm). *Pseudomonas aeruginosa* was affected by extracts of all four solvents (11–14 mm). The highest level of resistance among tested bacteria was observed in XDR-*S. typhi* – they reacted only to methanol

extract (9 mm). *S. epidermidis* was affected in methanol (14 mm), ethanol (11 mm), and diethyl ether (10 mm). MRSA did not demonstrate sensitivity to any papaya leaf extract.

Antifungal Activity

The antifungal test was performed against nine fungal species using the same disk diffusion technique. Most of the results were negative for all extract-fungi tested, but selectivity was observed in some of the samples from Custard apple (*A. squamosa*) and Orchid/Kachnar (*B.*

variegata) against *Candida* species. The results indicated that the inhibitory effect against *C. albicans* was observed for chloroform fraction of *B. variegata* (0.8 mm); the susceptibility of *C. tropicalis* was noted against *B. variegata* DEE (1.3 mm); while *C. kursii* was inhibited by *A. squamosa* EtOH (1 mm), DEE (0.9 mm) and *B. variegata*

MeOH (0.6 mm), CHCl₃ (0.7 mm) and DEE (0.9 mm); *C. lusitaniae* was inhibited by methanol (0.9 mm), CHCl₃ (0.9 mm), DEE (0.8 mm) and *B. variegata* MeOH (0.6 mm), EtOH (0.7 mm), CHCl₃ (1 mm), DEE (0.9 mm). No susceptibility was observed against *Aspergillus spp.* and *Rhizopus*, *Mucor*.

Table 4: Bactericidal effect of extracts from leaves of *Carica papaya*; zone of inhibition in mm; (–) = none.

Bacteria	Met	Eth	Chlo	Dieth	LE	FU	AZ	CEF	CE	DW
<i>S. typhi</i>	12	10	–	11	31	17	11	34	32	–
<i>XDR-S. typhi</i>	09	–	–	–	–	–	–	08	09	–
<i>S. aureus</i>	22	–	–	19	37	28	–	28	28	–
<i>MRSA</i>	–	–	–	–	–	–	–	–	–	–
<i>S. epidermidis</i>	14	11	–	10	34	–	–	–	–	–
<i>P. aeruginosa</i>	12	14	11	12	26	19	18	14	13	–
<i>K. pneumoniae</i>	–	10	–	–	30	7	–	36	28	–
<i>E. coli</i>	–	–	–	–	40	19	17	39	23	–
<i>Proteus mirabilis</i>	–	–	–	–	39	18	11	37	45	–
<i>Proteus vulgaris</i>	–	–	–	–	39	16	13	40	46	–
<i>Vibrio cholerae</i>	–	–	–	–	25	28	20	21	23	–
<i>S. pyogenes</i>	–	–	–	–	27	13	–	24	19	–

(Met): Methanol extract, (Eth): Ethanol extract, (Chl): Chloroform extract, (Die): Diethyl ether, (LE): Levofloxacin, (FU): Fusidic acid, (AZ): Azithromycin dihydrate, (CEF): Cefotaxime, Sodium, (CE): Ceftriaxone sodium

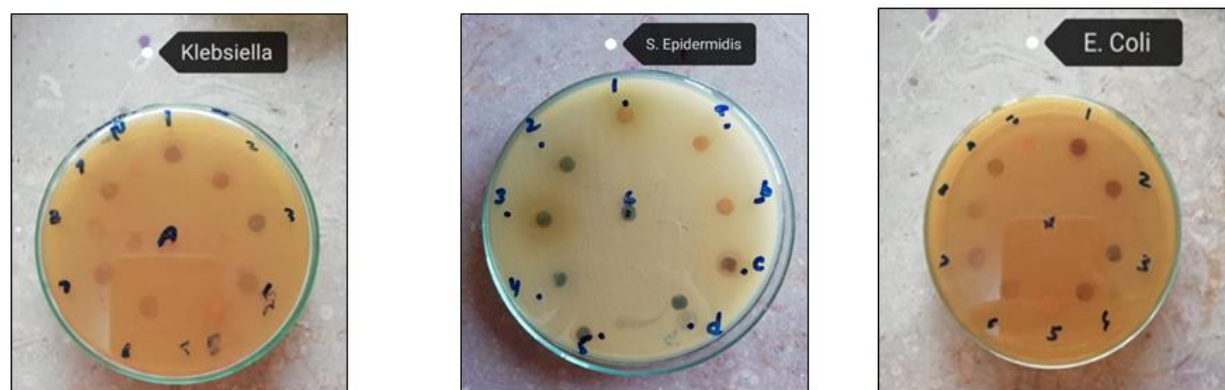


Figure 10: Inhibition zones formed by extracts of *Carica papaya* leaves on a disk diffusion plate.

Table 6: Antifungal activity of extracts of *Annona squamosa* (Custard Apple) and *Bauhinia variegata* (Orchid) against selected strains of fungi (Zone of inhibition in cm); (–) indicates no activity.

Fungus	Custard Apple MeOH	Custard Apple EtOH	Custard Apple CHCl ₃	Custard Apple DEE	Orchid MeOH	Orchid EtOH	Orchid CHCl ₃	Orchid DEE
<i>A. flavus</i>	–	–	–	–	–	–	–	–
<i>A. niger</i>	–	–	–	–	–	–	–	–
<i>A. fumigatus</i>	–	–	–	–	–	–	–	–
<i>T. mentagrophytes</i>	–	–	–	–	–	–	–	–
<i>Rhizopus sp.</i>	–	–	–	–	–	–	–	–
<i>Mucor sp.</i>	–	–	–	–	–	–	–	–
<i>C. albicans</i>	–	–	–	–	–	–	0.8	–
<i>C. tropicalis</i>	–	–	–	–	–	–	–	1.3

<i>C. glabrata</i>	—	—	—	—	—	—	—	—
<i>C. kursii</i>	—	1.0	—	0.9	0.6	—	0.7	0.9
<i>C. lusitaniae</i>	0.9	—	0.9	0.8	0.6	0.7	1.0	0.9



Figure 13: *C. lusitaniae* plate showing zone of inhibition with extracts of *Bauhinia variegata* and *Annona squamosa*.

Antifungal activity of extracts of *Codium fragile* and *Carica papaya*

In the additional dataset, antifungal activity of extracts of *C. fragile* and *C. papaya* was assessed on nine strains of fungi. Out of these only *A. niger* was found to be

susceptible to extracts of *C. fragile* (methanol 0.8 mm and diethyl ether 1.3 mm). No other strains such as other *Aspergillus*, *Candida spp.*, *Rhizopus*, and *Mucor* were found to be susceptible to any of the two types of extracts.

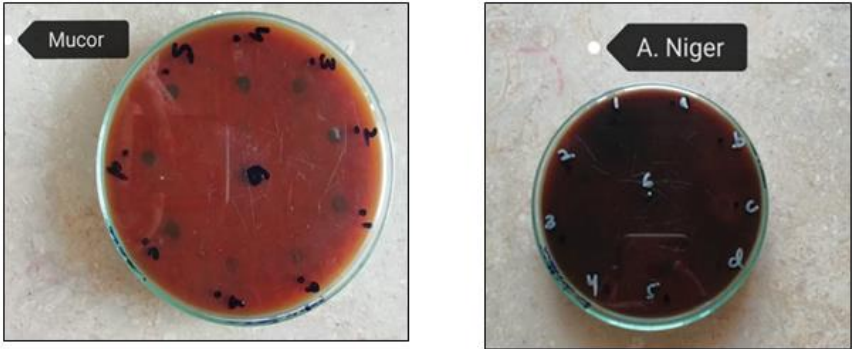


Figure 14: Disk diffusion plate for *Aspergillus niger* & *Mucor* inhibition zones with *Codium fragile* diethyl ether extract.



Figure 15: Fungal plate (*T. mentagrophytes*) with no inhibition zones (representative of limited antifungal activity).

Minimum Inhibitory Concentrations

All extracts showing positive inhibitory activity in the disc

diffusion test were further analyzed for their minimum inhibitory concentration (MIC) by using two-fold dilutions

(0.8, 0.4, 0.2 CFU/mL in DMSO). In all cases, the MIC was observed in the first dilution (0.8 CFU/mL), indicating that the active extract showed inhibition at the highest concentration but did not show inhibition at the lower concentrations (0.4 and 0.2 CFU/mL).

DISCUSSION

Overall, the findings of this study support the steadily increasing amount of evidence pointing at the promising antimicrobial properties of extracts from medicinal plants and marine algae. ; The latter being of particular importance due to the ongoing antibiotic resistance of numerous pathogens and lack of effective treatment measures. Several key points should definitely be highlighted.

First of all, the higher efficiency of *B. variegata* flower extracts compared to extracts from other plants used in this study can be attributed to its known antimicrobial properties and active ingredients. The genus *Bauhinia* (Fabaceae) is famous for the presence of high amounts of flavonoids, tannins, and phenols, which are able to affect bacterial cell membranes, chelate vital metal ions, and prevent pathogen's quorum sensing (Kuo et al., 2002; Patel & Kumar, 2008). It is also worth noting that *B. variegata* exhibited high efficiency in inhibiting growth of MRSA. The problem of MRSA is extremely common around the world, especially taking into account its status of a primary cause of nosocomial infections, and searching for non-beta-lactam-based therapeutic agents is an urgent issue in clinical microbiology (Boucher et al., 2009).

Though the activity exhibited by papaya leaf extracts was somewhat selective, it was nevertheless remarkable in some of the combinations. For instance, the large 22 mm inhibition zone of the methanol extract on *S. aureus* is one of the highest inhibition zones obtained in this research study and is comparable to those obtained in other studies conducted in the area (Ahmad et al., 2011). *Carica papaya* leaves contain bioactive components such as papain, carpaine, choline, and different types of phenolics, whose individual activities have been established to possess antimicrobial, anti-inflammatory, and antioxidant effects (Zunjar et al., 2016). This selectivity towards Gram-positive cocci as compared to Gram-negative rods found in most plant extracts used in antimicrobial studies may be attributed to cell wall differences that exist between Gram-negative and Gram-positive bacteria; the outer membrane in Gram-negative bacteria acts as another layer of barrier for the entry of hydrophilic phytochemicals.

Of all the outcomes reported from the experiments with marine algae, the antimicrobial effects observed on bacteria

appear to be most clinically interesting. Extracts of the *C. fragile* species exhibited antibacterial activity on susceptible and drug-resistant bacterial isolates, including XDR-*S. typhi*, which is known to be causing a typhoid epidemic in Pakistan that has been challenging public health authorities since 2016. Extensively drug resistant typhoid fever is caused by strains that have developed resistance to all approved first and second-line drugs, except for the antibiotic azithromycin (Klemm et al., 2018). The fact that the *C. fragile* ethanol fraction was able to inhibit an XDR-*S. typhi* isolate and form a zone of inhibition of 15mm in diameter in laboratory conditions deserves further investigation, including identification of the active compounds. It is known that this species is able to biosynthesize sulfated polysaccharides and acetylenic compounds of wide-ranging antimicrobial activity (Oh et al., 2010), but the exact substances responsible for the antibacterial effect observed in this case need to be identified.

In case of the antifungals, however, the effectiveness was rather limited. The specificity seen restricted only to *Candida* species and especially to the rare species *Candida kursii* and *C. lusitaniae* is an interesting observation. *Candida kursii* and *C. lusitaniae* are newly recognized opportunistic pathogens gaining importance in the clinic, especially for immunocompromised individuals, while the latter species is well-known for its resistance to the drug amphotericin B (Pfaffer & Diekema, 2007). Hence, the sensitivity of *Candida* species mentioned above to extracts from the plants *A. squamosa* and *B. variegata* even in lower concentrations needs to be investigated further. Finally, the absence of inhibitory effect of all studied samples against filamentous fungi (e.g., *Aspergillus species*, *Rhizopus*, and *Mucor*) is due to strong fungal antifungal defense systems in these microbes and poor phytochemical penetration through their heavily chitinized cell wall layers.

The universal absence of any inhibitory effect exhibited by the aqueous extracts from all test subjects (both bacteria and fungi) aligns with scientific literature indicating that the major antimicrobial compounds within these organisms have high lipophilicity and are better suited for extraction using organic solvents (Burt, 2004). In terms of potential applications, this indicates that extracts prepared using ethanol will have superior efficacy compared to aqueous extracts, even though the former can be easily prepared under conditions of limited resources. Furthermore, the fact that the MIC values of the effective extracts tend to cluster around 0.8 CFU/mL implies that they have a small concentration range.

However, the weaknesses of the current research must be highlighted. While disk diffusion tests give valuable preliminary insights into antibacterial effects, they cannot replace the more sophisticated methods of MIC measurement through broth microdilution, time-kill curve analysis, and animal testing. The precise amounts of the biologically active ingredients in the studied samples were not established, and the inconsistencies between the results obtained in different solvents confirm the necessity of a thorough fractionation process. The evaluation of the cytotoxic properties of active extracts should be also conducted.

CONCLUSION

In this study, it has been shown that the extracts of *Carica papaya*, *Annona squamosa*, *Bauhinia variegata*, and the marine algae *Codium fragile* from Pakistan are effective against a wide array of bacteria and have strong antimicrobial activity. From the plants, extracts of *Bauhinia variegata* flowers displayed the highest activity against bacteria, whereas papaya leaf extracts showed the best efficacy against *S. aureus*. Marine algae were also found to have significant antibacterial activity against both resistant and susceptible pathogens. Antifungal effects were also observed in some plant extracts; however, they were specific against clinically important *Candida species*.

It is apparent from the lack of any inhibitory activity in aqueous extracts that appropriate solvent must be selected for the phytochemical extractions in order to obtain antimicrobial substances. It appears that lipophilic phytochemical compounds are responsible for the antimicrobial activity. This work provides evidence that there are many local sources in Pakistan for obtaining antimicrobial substances, and thus, the country's biologic diversity is a good source for such natural drugs. Subsequent research efforts need to concentrate on bioassay-guided separation of active molecules, the determination of their mode of actions, synergy studies with existing antibiotics, and their biological activity and safety profile in animal models. If conducted properly, these plants and algae could provide promising avenues for the creation of innovative, cost-effective, and locally sourced antimicrobial agents.

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