Biocatalysis and Agricultural Biotechnology 57 (2024) 103123

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Antibacterial and anticancer activities of *Nymphaea nauchali* rhizome extracts

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ARTICLE INFO

Handling Editor: Dr. Ching Hou

Keywords:
Nymphaea nauchali rhizome
Antiproliferative
Pseudomonas
Antibacterial
Inner core

ABSTRACT

The present study was conducted to discover the antibacterial and anticancer activities of *Nymphaea nauchali* rhizome peel and core extracts. Antibacterial activity of ethanol, methanol, and acetone extracts of *N. nauchali* rhizome peel and inner core extracts has been tested against six biofilm-forming bacterial pathogens, *E. coli*, *P. aeruginosa*, *E. faecalis*, coagulase –ve *Staph. aureus*, *Staph. aureus*, and *P. vulgaris* by agar well diffusion assay. The size of inhibition zones around the wells in the presence of different extracts was used to determine the sensitivity of test bacteria. The anticancer activity of extracts was checked using the MCF-7 cell line, and potency was defined in terms of IC_{50} values. Methanol, ethanol, and acetone peel extracts showed more antibacterial activities against all test bacteria than inner core extracts. *E. faecalis* was highly resistant to acetone inner core extracts, and *P. aeruginosa* showed higher sensitivity to ethanol inner core extract. Ethanolic extracts of rhizome peel and inner core were more effective against the MCF-7 cell line than methanol and acetone extracts. IC_{50} values of 208.20 μgml^{-1}and 136.16 μgml^{-1}were noted for peel and inner core ethanol extracts, respectively. *N. nauchali* rhizome can be used as a potential entrant in developing novel antibacterial and anticancer phytoformulations.

1. Introduction

Plants with medicinal value have been used since from pre-historic era in India and Chinese, Egyptian, European, and Mediterranean cultures. According to the world health organization (WHO) estimates, 80% of the worldwide population depends on herbal remedies to satisfy their primary healthcare needs. In developing countries such as India and well-developed countries like the United States, plant-based drugs hold 80% and 25 % share of available drugs (National Health Portal, 2016; www.nhp.gov.in, N.H.P, 2016). The human population is continuously exposed to health challenges of emerging drug-resistant strains, further intensified due to the inefficacy of available drugs and organisms' behaviour to escape the host's defence mechanisms. Hence, search for novel, safe, and bioactive molecules continues and is an area of growing interest for research and the scientific fraternity. *Nymphaea nauchali,* or white Lily, a member of the Nympheaceae family, is an aquatic herb available in different world regions. The general medicinal uses of this plant have been known in Ayurveda and Siddha systems, particularly for the treatment of urinary and liver disorders, diabetes, in-

Abbreviations: RP, rhizome peels; RIC, rhizome inner core; RPE, rhizome peels ethanol extract; RPM, rhizome peels methanol extract; RPA, rhizome peels acetone extract; RICE, rhizome inner core ethanol extract; RICM, rhizome inner core methanol extract; RICA, rhizome inner core acetone extract.

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https://doi.org/10.1016/j.bcab.2024.103123
Received 13 September 2023; Received in revised form 27 September 2023; Accepted 18 March 2024
Available online 19 March 2024

flammation, tumour, and gastric disorders (Dash et al., 2013; Parimala Mabel and Shoba, 2014; Rajagopal et al., 2008; Sarma and, 2008; Sikder et al., 2012). Different parts of *N. nauchali,* including flowers and seeds, have been studied earlier and reported as having tannins, flavonoids, phenols, glycosides, and sterols (Dash et al., 2013; Parimala Mabel and Shoba, 2014). The *N. nauchali* has gained worldwide attention due to the presence of these phytometabolites and their antibacterial, antioxidant, antiproliferative and antidiabetic potential (Huang et al., 2012, 2010; Jahan, I. et al., 2012; Mohan Maruga Raja MK et al., 2012). Recently Anand et al. (2021)reported the anti-hyperglycaemic and antioxidant capacity of boiled rhizome powder of *N. nauchali* based on its phytoconstituents analysis and indicated its role in managing oxidative stress and hyperglycemia (Anand et al., 2021). However, this plant's rhizome remained under investigation regarding its antimicrobial and anticancer potential. The present study evaluated ethanol, methanol, and acetone extracts of rhizome peel and inner core against a few biofilms forming bacterial pathogens and MCF-7 cells.

2. Materials and methods

2.1. Plant collection

Healthy rhizomes of the *N. nauchali* plant were collected from Patanwada village, GadChiroli, Maharashtra, India, in April 2022. The plant was authenticated by Dr Vasant Kahalkar, Department of Botany, Mahatma Gandhi Arts, Science, and Late N. P. Commerce College, Armor, with a voucher number of the specimen (BNL1).

2.2. Extraction of phytoconstituents

Clean, dust-free, and fresh rhizomes were collected and peeled to separate rhizome peels from the inner core. Both rhizome peels (RP) and rhizome inner core (RIC) parts were dried in the shade for 7–10 days and ground separately in a grinder to obtain fine RP and RIC powders. Ethanol, methanol, and acetone extracts of RP (RPE, RPM, and RPA) and RIC (RICE, RICM, and RICA) were prepared by mixing respective solvents and powdered rhizome parts in 1:30 ratio and keeping the mixture under shaking conditions for 24–48 h at 120 rpm at room temperature. The individual mixtures were then filtered and evaporated to dryness using a rotary evaporator (Fig. 1). The dried extracts were diluted in 10% dimethyl sulfoxide (DMSO) to final concentrations of 100 μgml^{-1} and 250 μgml^{-1}.

2.3. Test organisms

Six clinical bacteria, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, coagulase-negative *Staphylococcus aureus* (CONS), *Enterococcus faecalis*, and *Proteus vulgaris* were procured from Department of Microbiology, Shankarrao Chavan Government Medical College, Vishnupuri, Nanded and used in the present study.

2.4. Biofilm formation potential of test bacteria

The biofilm formation potential of all test bacteria was evaluated by microtiter plate assay (O'Toole, 2010), and the bacteria were classified as weak, moderate, and strong biofilm formers according to the method followed by Hassan et al. (2011).

$$ODcut = ODavg.\ ofnegativecontrol + 3 \times standarddeviation\ (SD)\ \ ofODsofnegativecontrol$$

Biofilm could be strong, moderate, or weak if;

1 OD ≤ ODcut (Non-biofilm-former)
2 ODcut < OD ≤ 2 × ODcut (Weak biofilm-former)
3 2 × ODcut < OD ≤ 4 × ODcut (Moderate biofilm-former)
4 OD > 4 × ODcut (Strong biofilm-former)

2.5. Screening of extracts for phytoconstituents

The extracts were analyzed for alkaloids, carbohydrates, reducing sugars, glycosides, flavonoids, phenolic compounds, tannins, saponins, phytosterols, lignin, anthocyanins, and resins using standard protocols (Junaid and Patil, 2020).

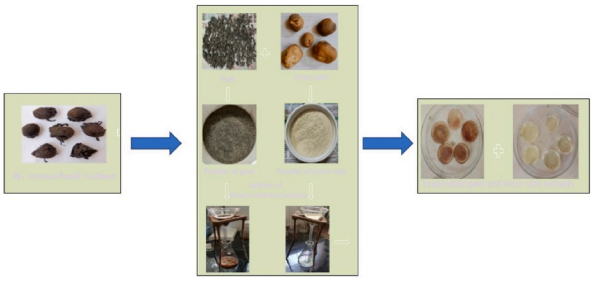

Fig. 1. Preparation of *N. nauchali* rhizome RP and RIC extracts.

2.6. Antibacterial activity of extracts

The antibacterial effect of RPE, RPM, RPA, RICE, RICM, and RICA extracts on test bacteria was determined by agar well diffusion assay (Daoud et al., 2015). The test bacteria were grown individually at 37 °C for 24 h in Luria broth (HiMedia) to attain growth corresponding to 0.5 McFarland turbidity standards. The individual inoculums were spread onto the solidified sterile Mueller Hinton agar surfaces. The wells of 5 mm were punctured on agar medium, and individual extracts were added separately into the wells. The plates were kept for diffusion at 4 °C for 30 min and incubated at 37 °C for 24 h. After incubation, the plates were observed for inhibition zones measured in millimeters. The sizes of inhibition zones were used to determine the sensitivity of test bacteria toward individual extracts. Ampicillin (10 $\mu g ml^{-1}$) and methicillin (30 $\mu g ml^{-1}$) were standard antibiotics for Gram-negative and Gram-positive test bacteria. DMSO (10%) and sterile distilled water were used as a solvent and negative control, respectively. The test was carried out in triplicate, and results were recorded as mean ± standard deviation.

2.7. Anticancer activity of extracts

Human breast adenocarcinoma (MCF-7) cell line was procured from National Centre for Cellular Sciences (NCCS), Pune and cultivated in Dulbecco's Modified Eagle (DMEM) high glucose media (Gibco), supplemented with foetal bovine serum (10%), and 1% antibiotic-antimycotic solution at 37 °C and in the presence of 5% CO_2 and 18%-20%O_2 in a CO_2 incubator. The cytotoxicity effect of RPE, RPM, RPA, RICE, RICM, and RICA extracts was studied using 3-(4,5- dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Siddiqui et al., 2008). Doxorubicin (10 $\mu g ml^{-1}$) was used as positive control; medium without cells was medium control, and medium with cells without extract or standard drug was kept as a negative control. The extracts which showed more than 50% inhibition at 250 $\mu g ml^{-1}$ were selected further to study their effect at varying concentrations and to determine IC_{50} values. The % cell viability in the presence of extracts was determined as

$$\%\ \text{viable cells} = \frac{OD_{570} of treated cells}{OD_{570}\ of untreated cells} \times 100$$

The IC_{50} values were determined using a linear regression equation (Y = mx +c) where Y = 50, m, and c values were derived from the viability graph.

3. Results

3.1. Phytochemical analysis

The phytochemical analysis of RPE, RPM, RPA, RICE, RICM, and RICA extracts is shown in (Table 1). All peel extracts showed the presence of alkaloids, reducing sugar, glycosides, phenolic compounds, tannins, saponins, flavonoids, phytosterols, lignin, anthocyanins and resins. In contrast, carbohydrates, alkaloids, anthocyanins, resins, phytosterols, and amino acids, were not found in inner core extracts. All extracts showed the variable presence of phenolic compounds, with higher concentrations being detected in RPE extracts. Flavonoids are detected in RPE, RPM, RICE, and RICM; the highest concentrations are found in RICE extracts. Ninhydrin test for proteins and amino acids was positive only with RPE extracts.

3.2. Biofilm formation potential of test bacteria

Among all tested bacteria, *P. aeruginosa*, *Staph. aureus* and CONS are strong biofilm formers, *P. vulgaris* is moderate biofilm former, and *E. coli*, and *E. faecalis*, are weak biofilm formers (Table 2).

Table 1
Phytochemical analysis of RPE, RPM, RPA, RICE, RICM, and RICA extracts.

Sr. No.	Test	Observation					
		RPE	RPM	RPA	RICE	RICM	RICA
1.	Alkaloid	++	+	+	_	_	_
2.	Carbohydrates	+	+	+	+	_	_
3.	Reducing sugars	++	+ +	+ +	+ +	+ +	+ +
4.	Glycosides	+	+	+	+	+	++
5.	Cardiac glycosides	_	_	_	_	_	_
6.	Proteins and amino acids	+	_	_	_	_	_
7.	Flavonoids	+ +	+	_	++ +	+	_
8.	Phenolic compounds	++ +	++	++	++	++	+
9.	Tannins	++	++	++	++	++	+ +
10.	Phlobatamins	+	+	+	_	_	_
11.	Saponins	++	++	++	+	+	_
12.	Phytosterols	++	+	+	_	_	_
13.	Lignin	++	++	+	+	+	+
14.	Anthocyanins	+	+	+	_	_	_
15.	Resin	+	++	+	_	_	_

(+ weak presence, ++ moderate presence, +++ strong presence, and − absent or not found).

Table 2
Degree of biofilm formation in test organisms.

Name of Bacteria	Degree of biofilm formation
Escherichia coli	WEAK
Proteus vulgaris	MODERATE
Pseudomonas aeruginosa	STRONG
Enterococcus faecalis	WEAK
Staphylococcus aureus	STRONG
coagulase-negative *Staphylococcus aureus* (CONS)	MODERATE

3.3. Antibacterial activities of extracts

The effect of different extracts of *N. Nauchali* rhizome against test bacteria is shown in (Table 3). In general, peel extracts showed noticeable antibacterial activity against all test bacteria compared to inner core extracts. The growth of Gram-negative biofilm-forming *P. aeruginosa* was inhibited more in the presence of RPA (18 ± 0.21 mm) followed by RPM (16 ± 0.05 mm) and RPE (15.5 ± 0.01 mm). RPE was more effective against *Staph. aureus* (16 ± 0.026 mm) and CONS (14.2 ± 0.06 mm) were Gram-positive strong biofilm-forming bacteria. The growth of *P. vulgaris*, a Gram-negative, moderate biofilm-forming bacterium and *E. coli,* a Gram-negative, weak biofilm former, were inhibited indifferently in the presence of all peel and inner core extracts. The RPE, RPM, and RPA had higher inhibition zones of 12 ± 0.032 mm, 13 ± 0.046 mm, and 13.5 ± 0.012 mm against *E. faecalis*. Among three inner core extracts, RICA produced the highest zone of inhibition (14 ± 0.02 mm) against CONS and *Staph. aureus* (12.5 ± 0.16 mm) respectively. Among Gram-negative test bacteria, *P. aeruginosa* and *P. vulgaris* showed resistance to ampicillin at the studied concentration, whereas *E. coli* showed moderate sensitivity (12 mm) towards ampicillin. All Gram-positive test bacteria were resistant to methicillin at 30 μgml^{-1}.

3.4. Anticancer activity

Cytotoxic activity of *N. nauchali* rhizome peel and inner core extracts against MCF-7 cell line was assessed using MTT assay initially at 250 μgml^{-1}. Only RPE and RICE effectively reduced viable cell count to 50% at initial concentrations (data not shown) and hence, selected further at varying concentrations (50, 100, 200, 400, 1000 μgml^{-1}) to determine IC_{50} values. The MCF-7 viable count was decreased in a dose-dependent manner in the presence of RPE and RICE at studied concentrations. The viability of MCF-7 was not affected much at 50 μgml^{-1} of extracts. The MCF-7 cells exposed to higher concentrations of RPE and RICE extracts were more effective in reducing cell viability. At 400 μgml^{-1} and 1000 $\mu gml^{-}1$, RPE and RICE altered typical morphology and reduced the size of MCF-7 cells. RICE extract was more cytotoxic (IC_{50} = 136.16 μgml^{-1}) against MCF-7 compared to RPE extract (IC_{50} = 208.20 μgml^{-1}, Fig. 2 & Fig. 3).

4. Discussion

Bacterial biofilms and related infections threaten human health globally (Tasneem et al., 2018),as more than 60% of device and non-device-related bacterial infections are linked with biofilm formation (Lewis, 2001). Treatment of biofilm-associated bacterial infections is challenging due to their effect on penetration and availability of antibiotics resulting in increased resistance to antimicrobial compounds (Iwamoto et al., 2010). In addition to challenges in treating biofilm-mediated infections, breast cancer is the second root cause of death among women worldwide. According to the WHO, 2.3 million breast cancer cases were reported in 2020, of which 6,85,000 deaths were recorded. Keeping in view the higher incidence of multidrug resistance among infectious bacteria and cancer cells, the increasing economic burden and load for the treatment, and the need for natural, safe, and cost-effective bioactive molecules, we selected *N. nauchali* rhizome as a source of the antibacterial and cytotoxic molecule.

In this study, RP extracts showed higher activity against strong biofilm-forming bacteria such as *P. aeruginosa*, *Staph. aureus* and CONS are significant in hospital-acquired and community-acquired infections (Khan et al., 2015). RICA extract was more effective in controlling the growth of *Staph. aureus* and CONS. Although peel and inner core *N. nauchali* rhizome inhibited the growth of test bacteria, peel extracts were highly potent compared to inner core extracts. The variation in activity may be due to the type and extent of phytoconstituents present in extracts. Peel extracts showed more presence of alkaloids, phenols, and flavonoids, which may be attrib-

Table 3
Antibacterial activity of *N. nauchali* rhizome extracts against test bacteria.

Bacterial strains	Extracts/standard antibiotic zone of inhibition (mm)							
	RPE	RPM	RPA	RICE	RICM	RICA	Ampicillin (10 μgml^{-1})	Methicillin (30 μgml^{-1})
P. aeruginosa	15.5 ± 0.011	16 ± 0.05	18 ± 0.21	11 ± 0.12	11 ± 0.51	11 ± 0.35	R	–
P. vulgaris	11 ± 0.27	10 ± 0.201	9.5 ± 0.29	9.5 ± 0.612	9 ± 0.42	9 ± 0.01	R	–
Staph. aureus	16 ± 0.26	11 ± 0.072	11 ± 0.21	9.5 ± 0.16	9 ± 0.022	12.5 ± 0.51	–	R
E. faecalis	12 ± 0.32	13 ± 0.046	13.5 ± 0.012	9 ± 0.18	10 ± 0.11	–	–	R
E. coli	8.5 ± 0.06	10.5 ± 0.46	10.5 ± 0.33	9.5 ± 0.19	9.5 ± 0.03	8.5 ± 0.05	12	–
CONS	14 ± 0.06	10 ± 0.76	12 ± 0.62	7 ± 0.032	7.5 ± 0.036	14 ± 0.02	–	R

(R: resistant, -: not tested).

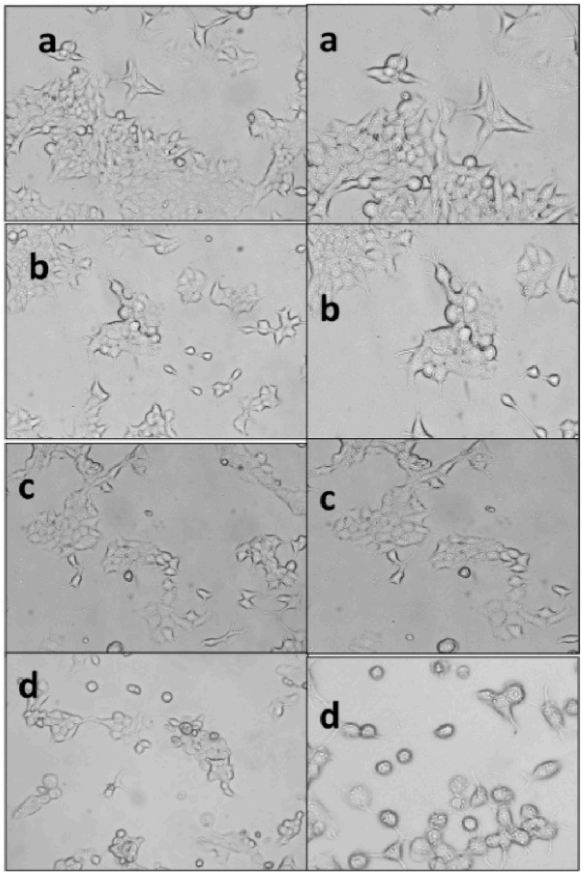

Fig. 2. Effect of varying concentrations of RPE (A lane) and RICE (B lane) extracts of *N. nouchali* rhizome on the viability of MCF-7 cell line (a: 100 μgml-1, b: 200 μgml-1, c: 400 μgml-1, d: 1000 μgml-1).

uted to the higher antibacterial effect of peel extracts (Sarma and and, 2008).Antibiotic-resistant infectious bacteria pose a serious threat and global health challenge. According to WHO, we are approaching to "post-antibiotic era" in which many people die due to the inefficacy of drugs for treating even minor infections (Harbarth et al., 2015; Parimala and Shoba, 2014). The methicillin-resistant *Staph aureus* (MRSA) is an essential group of multidrug-resistant bacteria that puts a huge economic burden and challenges the treatment of infectious diseases caused by them throughout the globe. Since ancient times, medicinal plants have significantly treated various diseases and disorders. In the present era of modern technology, too, we are highly dependent on them for primary health care. They are also significant sources for finding better alternatives for conventionally used antibiotics. In this regard, *N. nauchali* rhizome extracts towards which these antibiotic-resistant bacteria showed sensitivity could be a highlighting aspect. These extracts can be explored further to develop natural bioactive molecules against ampicillin and methicillin-resistant bacteria. *N. nauchali* is a storehouse of secondary metabolites, including saponins, tannins, flavonoids, phenols, and phytosterols. Earlier studies documented the isolation of these metabolites from different parts of the plant, like a flower (Dash et al., 2013), and phenolic compounds like quercetin, catechin, and gallic acid from seed (Parimala and Shoba, 2014).The pharmacological activities, including antimicrobial and anticancer effects of flower and seed extracts of members of the Nymphaeaceae family, including *N. nauchali, N. alba, N. lotus,* and *N. Stellata,* have been reported earlier (Cudalbeanu et al., 2018; Iqbal et al., 2018; Parimala and Shoba, 2014). Recently Dias et al. (2021) reported the antioxidant and food preservation potential of *N. nauchali* petal extract and indicated it as an excellent nutraceutical source for the food industry(Dias et al., 2021). However, there is little evidence of the antimicrobial and cytotoxic effect of *N. nauchali* rhizome peel and core extracts. The cytotoxic effect of RPE and RICA extract against MCF-7 cells also indicated their promising potential in developing antiproliferative drugs. The inhibition of MCF-7 cell growth may correlate to flavonoids in the extracts, which are known to arrest various cell cycle phases. They also regulate apoptotic signalling pathways' expression and inhibit cell metastasis and proliferation(Gupta et al., 2019; Iqbal et al., 2018). At higher concentrations of RPE and RICE, changes in the morphology of MCF-7 cells were observed, and such changes as a possible cause of cell death are in agreement with past studies (Berrington and Lall, 2012; Farshori et al., 2013).Considering the drug resistance associated with chemotherapeutic drugs, plant extracts containing valuable biomolecules can be more selective and safer.

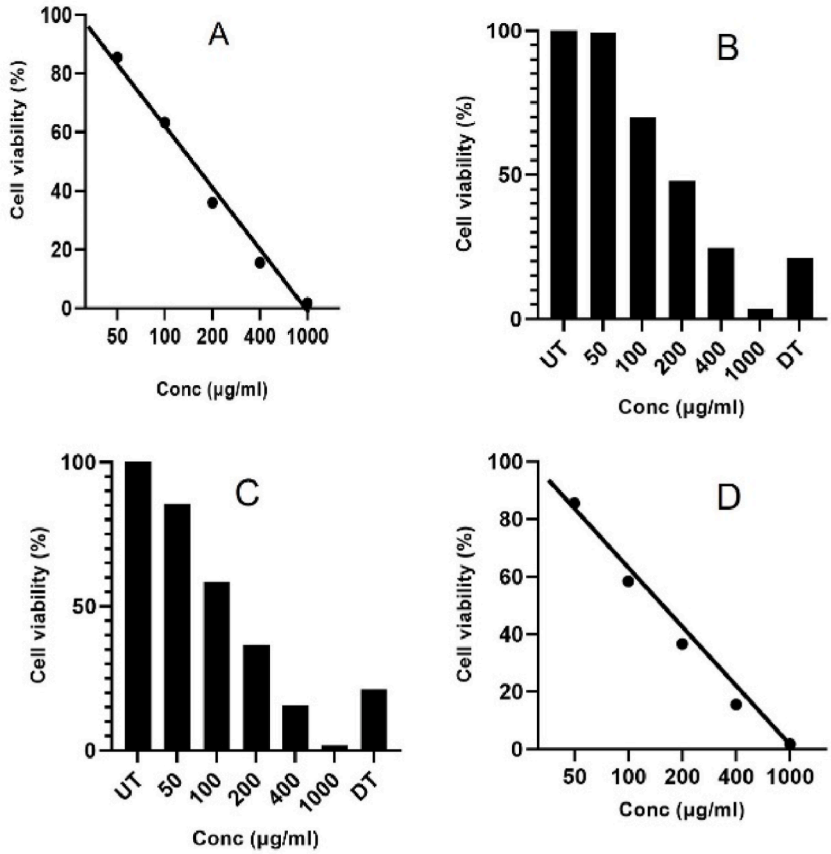

Fig. 3. Effect of varying concentrations of RPE (A and B), RICE (C and D) and Doxorubicin (10 µgml-^{1}) on the viability of MCF-7 Cells after 24 h incubation (UT – Untreated and DT – in the presence of doxorubicin tenµgml^{-1}).

5. Conclusion

The present study indicated the antibacterial and anticancer potential of *N. nauchali* rhizome extracts. The strong biofilm forming bacterial pathogens including ampicillin resistant *Pseudomonas aeruginosa* and methicillin resistant *Staphylococcus aureus* are clinically important due to their involvement in various community and hospital acquired infections. The ethanolic rhizome extract of *N. nauchali* showed considerable activity against biofilm forming bacteria and MCF-7 cells. It provided new insights for detailed future investigation for their use in breast cancer and infectious disease therapy.

Authors' contributions

"All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Bhagyashri Lanjewar], [Amani Esmail], [Rania N. Ghaleb], [Mujahed Siddiqui], and [Sunil Jadhav]. The final draft of the manuscript was written by [Bhosale Hemlata], and all authors commented on previous versions. All authors read and approved the final manuscript."

Funding

"This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors."

Declaration of competing interest

All authors declare that there's no financial/personal interest or belief that could affect their objectivity and there are no potential competing interest to declare.

Data availability

Data will be made available on request.

Acknowledgments

The authors would like to thank DST- FIST Sponsored School of Life Sciences, Swami Ramanand Teerth Marathwada University, Nanded, MS, India, and Taiz University for providing support and facilities to conduct this research work.

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