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Crinum asiaticum* L. bulb extracts as a potential source of novel antimicrobial agents: An *in-vitro* study*K. Gowthaman^{*1}, P. Prakash¹, V. Ambikapathy¹, S. Babu¹, A. Panneerselvam²**¹PG and Department of Botany, A.V.V.M. Sri Pushpam College (Autonomous),

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ABSTRACT

This study evaluated the antimicrobial potential of *C. asiaticum* bulb extracts from three different areas (KGVA 1, KGVA 2, and KGVA 3) against clinically relevant bacteria and fungi. The aqueous and hydrated methanol extracts of *C. asiaticum* bulb showed broad-spectrum antibacterial activity against certain bacterial species, including *Escherichia coli*, *Proteus* sp, and *Pseudomonas aeruginosa*. The hydrated methanol extract of KGVA 2 showed significant antibacterial activity against *Pseudomonas aeruginosa*, with a zone of inhibition of 22.7 ± 0.29 mm at 80 μ l. The aqueous extract of KGVA 3 exhibited broad-spectrum antifungal activity against *Aspergillus* species, including *A. flavus*, *A. fumigatus*, and *A. niger*. The hydrated methanol extract of KGVA 1 showed significant antifungal activity against *A. niger*, with a zone of inhibition of 25.1 ± 0.15 mm at 80 μ l. These findings suggest that *C. asiaticum* bulb extracts have potential antimicrobial properties, making them a promising source for the development of novel antimicrobial agents.

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INTRODUCTION

The uses of medicinal plants have become increasingly important in modern healthcare, with many species being utilized in traditional medicine, folk remedies and the development of pharmaceuticals. The demand for these plants can transcend local markets and extend to national and international trade networks (Meerow and Snijman, 2006). One plant family that has garnered significant attention for its medicinal properties is the Amaryllidaceae family, a group of monocotyledonous plants found worldwide (Habartova *et al.*, 2016). This family is renowned for its distinctive alkaloid compounds and unique structural characteristics which have been found to possess a wide range of biological activities with potential therapeutic applications (Nair and Van Staden, 2020).

The Amaryllidaceae family is divided into three subfamilies and comprises approximately 80 genera and 1,200 species, primarily found in tropical, subtropical and warm temperate regions (Ronsted *et al.*, 2012; Christenhusz *et al.*, 2017). The *Crinum* genus, a member of the Amaryllidaceae family, has a rich history of classification and distribution. With its origins dating back to the 18th century, the genus has undergone significant revisions, resulting in the recognition of over 100 species worldwide (Linnaeus, 1753; Lekhak *et al.*, 2015; Lekhak and Yadav, 2012).

The *Crinum* genus, comprising antimalarial, antiviral, anti-tumor and anti-diabetic activities (Jagtap *et al.*, 2014; Swati *et al.*, 2021). *Crinum asiaticum*, commonly known as the Asian *Crinum* lily, is a traditional medicinal plant that has been used for centuries in various parts of the world, particularly in Asia and Africa (Danquah *et al.*, 2022; Barile *et al.*, 2005). *Crinum asiaticum* has been used in folk medicine for its diverse therapeutic properties including anti-inflammatory, antipyretic and antimicrobial activities (Chase *et al.*, 2009; Takos and Rook, 2013). The plant's bulb, leaves and flowers have been used to treat various ailments, such as fever, rheumatism and skin infections (Mahomodally *et al.*, 2021).

The bulb of *C. asiaticum* has been found to possess significant antimicrobial against a range of microorganisms including *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*, highlighting their potential as a natural antimicrobial agent (Goswami *et al.*, 2020). The findings of this study contribute to the understanding of the phytochemical composition and biological activities of *Crinum asiaticum* bulb extracts and provide valuable information for the development of new therapeutic agents from this plant. Additionally, the study validates the traditional uses of *Crinum asiaticum* and to promote the conservation and sustainable use of this valuable medicinal plant.

MATERIALS AND METHODS

Collection of plant material

Crinum asiaticum bulb (KGVA 1, KGVA 2 and KGVA 3) samples were sourced from three different places of Thanjavur, Nagappattinam and Namakal, Tamil Nadu, India.

Extraction of *C. asiaticum* bulb extracts

The extractions were performed at a temperature of 40°C for duration of 45 minutes, with constant magnetic stirring and a sample-to-solvent ratio of 1:20 (mass-to-volume). A total of two distinct solvents of aqueous, hydrated methanol were tested. Following extraction, the resulting samples were subjected to rotary evaporation, lyophilization and subsequent dilution with ultrapure water at the time of analysis. An augmented simplex centroid mixture design, comprising 10 unique solvent combinations was employed to assess the individual, binary and ternary effects of the solvents used (Elhami *et al.*, 2022).

Antimicrobial activity of *C. asiaticum* extracts

The antibacterial activity of *C. asiaticum* bulb (KGVA 1, KGVA 2 & KGVA 3) aqueous and hydrated methanol extracts were evaluated using a method described previously (Bauer, 1996). Aqueous and hydrated methanol extracts of *C. asiaticum* bulb (KGVA 1, KGVA 2 & KGVA 3) (20, 40, 60 and 80µl) were tested against Gram-positive bacteria *Bacillus subtilis* (MTCC 8297),

as well as three Gram-negative bacteria: *Escherichia coli* (MTCC 7410), *Proteus* sp. (MTCC 11803) and *Pseudomonas aeruginosa* (MTCC 3541). The bacterial strains were obtained from the Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh. The agar well diffusion method was employed for the antibacterial activities were determined with the diameter of the zone of inhibition (mm) measured and recorded.

The antifungal activity of *C. asiaticum* bulb (KGVA 1, KGVA 2 & KGVA 3) aqueous and hydrated methanol extracts were assessed using the agar well diffusion method against fungal strains such as *Aspergillus flavus* (MTCC 3518), *A. fumigatus* (MTCC 3070), *A. niger* (MTCC 13374) and *A. terreus* (MTCC 2578). The zone of inhibition formed around the well was measured and recorded.

Statistical analysis

The experiments were conducted in triplicate and the results were represented as mean values accompanied by their standard deviations.

RESULTS

Antimicrobial activity of *C. asiaticum*

The antibacterial potential of 3 different areas of *C. asiaticum* bulb extracts (KGVA 1, KGVA 2 and KGVA 3) extracts of aqueous and hydrated methanol were performed against a range of clinically relevant bacteria and fungi.

Antibacterial activity

Assessment of antibacterial potential of C. asiaticum Bulb (KGVA 1) against clinically relevant bacteria

The *Bacillus subtilis* was observed with 5.00±0.00, 8.00±0.01 and 9.00±0.30mm zone of inhibitions at 40, 60 and 80µl of *C. asiaticum* KGVA 1 extract. Similar concentrations expressed 8.70±0.21, 12.8±0.36 and 11.0±0.32mm zone of growth inhibitions were observed against *E. coli*. The *Proteus* sp. growth was inhibited with 4.90±0.11, 6.00±0.23 and 9.70±0.26mm at 40, 60 and 80µl of *C. asiaticum* KGVA 1 extract. The 3.20±0.00 and 4.50±0.01mm growth inhibition was observed from *Pseudomonas*

aeruginosa at 60 and 80µl concentration of bulb extract (Table 1).

Table 1. Assessment of antibacterial potential of *C. asiaticum* bulb (KGVA 1) aqueous extract against clinically relevant bacteria

| Name of the bacteria | Zone of inhibition (mm) | | | |
|----------------------|-------------------------|-----------|-----------|-----------|
| | 20 µl | 40 µl | 60 µl | 80 µl |
| <i>B. subtilis</i> | - | 5.00±0.00 | 8.00±0.01 | 9.00±0.30 |
| <i>E. coli</i> | - | 8.70±0.21 | 12.8±0.36 | 11.0±0.32 |
| <i>Proteus</i> sp | - | 4.90±0.11 | 6.00±0.23 | 9.70±0.26 |
| <i>P. aeruginosa</i> | - | - | 3.20±0.00 | 4.50±0.01 |

The values are expressed with Mean ± Standard deviation

Table 2. Assessment of antibacterial potential of *C. asiaticum* bulb (KGVA 1) hydrated methanol extract against clinically relevant bacteria

| Name of the bacteria | Zone of inhibition (mm) | | | |
|----------------------|-------------------------|-----------|-----------|-----------|
| | 20 µl | 40 µl | 60 µl | 80 µl |
| <i>B. subtilis</i> | - | - | 16.5±0.06 | 17.0±0.02 |
| <i>E. coli</i> | 11.6±0.30 | 15.6±0.11 | 15.9±0.28 | 16.0±0.00 |
| <i>Proteus</i> sp | 9.50±0.36 | 10.0±0.00 | 13.5±0.32 | 15.6±0.31 |
| <i>P. aeruginosa</i> | 4.20±0.33 | 5.20±0.74 | 6.70±0.12 | 9.60±0.22 |

The values are expressed with Mean ± Standard deviation

The antibacterial potential of *C. asiaticum* bulb (KGVA 1) hydrated methanol extract was evaluated against four clinically relevant bacteria. The *Bacillus subtilis* was observed with 16.5±0.06 and 17.0±0.02mm zone of inhibitions at 60 and 80µl of *C. asiaticum* KGVA 1 extract. The 11.6±0.30, 15.6±0.11, 15.9±0.28 and 16.0±0.00mm zone of inhibitions were observed against *E. coli* at 20, 40, 60 and 80µl concentration of bulb extract. The *Proteus* sp. growth was inhibited with 9.50±0.36, 10.0±0.00, 13.5±0.32 and 15.6±0.31mm by 20, 40, 60 and 80µl concentration of bulb extract. The 4.20±0.33, 5.20±0.74, 6.70±0.12 and 9.60±0.22mm growth inhibition was observed from *Pseudomonas aeruginosa* at 20, 40, 60 and 80µl concentration of bulb extract (Table 2).

Assessment of antibacterial potential of C. asiaticum Bulb (KGVA 2) against clinically relevant bacteria

The antibacterial potential of *C. asiaticum* bulb (KGVA 2) aqueous extract was evaluated against four clinically relevant bacteria. The results showed that

the aqueous extract exhibited antibacterial activity against *Proteus* sp, with a zone of inhibition of 9.31 ± 0.11 mm at $20 \mu\text{l}$ and 12.0 ± 0.00 mm at $40 \mu\text{l}$. However, no antibacterial activity was observed against *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* at both $20 \mu\text{l}$ and $40 \mu\text{l}$ concentrations. These findings suggest that the aqueous extract of *C. asiaticum* bulb (KGVA 2) has selective antibacterial activity against certain bacterial species, specifically *Proteus* sp. (Table 3).

Table 3. Assessment of antibacterial potential of *C. asiaticum* bulb (KGVA 2) aqueous extract against clinically relevant bacteria

| Name of the bacteria | Zone of inhibition (mm) | | | |
|----------------------|-------------------------|------------------|------------------|------------------|
| | 20 μl | 40 μl | 60 μl | 80 μl |
| <i>B. subtilis</i> | - | - | - | - |
| <i>E. coli</i> | - | - | - | - |
| <i>Proteus</i> sp | - | - | 9.31 ± 0.11 | 12.0 ± 0.00 |
| <i>P. aeruginosa</i> | - | - | - | - |

The values are expressed with Mean \pm Standard deviation

The antibacterial potential of *C. asiaticum* bulb (KGVA 2) hydrated methanol extract was evaluated against four clinically relevant bacteria. The results showed significant antibacterial activity against *Pseudomonas aeruginosa*, with zones of inhibition of 9.60 ± 0.26 , 16.7 ± 0.22 , 12.3 ± 0.25 and 22.7 ± 0.29 mm at 20, 40, 60 and $80 \mu\text{l}$. The extract also exhibited activity against *E. coli*, with zones of inhibition of 12.3 ± 0.31 mm at $20 \mu\text{l}$ and 14.5 ± 0.25 mm at $40 \mu\text{l}$.

Table 4. Assessment of antibacterial potential of *C. asiaticum* bulb (KGVA 2) hydrated methanol extract against clinically relevant bacteria

| Name of the bacteria | Zone of inhibition (mm) | | | |
|----------------------|-------------------------|------------------|------------------|------------------|
| | 20 μl | 40 μl | 60 μl | 80 μl |
| <i>B. subtilis</i> | - | - | - | - |
| <i>E. coli</i> | - | - | 12.3 ± 0.31 | 14.5 ± 0.25 |
| <i>Proteus</i> sp | - | 5.11 ± 0.00 | 10.1 ± 0.01 | 12.6 ± 0.22 |
| <i>P. aeruginosa</i> | 9.60 ± 0.26 | 12.3 ± 0.25 | 16.7 ± 0.22 | 22.7 ± 0.29 |

The values are expressed with Mean \pm Standard deviation

Additionally, activity was observed against *Proteus* sp, with zones of inhibition of 5.11 ± 0.00 , 10.1 ± 0.01 and 12.6 ± 0.22 mm at 40, 60 and $80 \mu\text{l}$. However, no

antibacterial activity was observed against *Bacillus subtilis* at both concentrations. These findings indicate that the hydrated methanol extract of *C. asiaticum* bulb (KGVA 2) has broad-spectrum antibacterial activity against certain clinically relevant bacterial species (Table 4).

Assessment of antibacterial potential of *C. asiaticum* Bulb (KGVA 3) against clinically relevant bacteria

The antibacterial potential of *C. asiaticum* bulb aqueous extract was evaluated against four clinically relevant bacteria. The results showed significant antibacterial activity against *E. coli*, with zones of inhibition of 12.5 ± 0.36 mm at $20 \mu\text{l}$, 15.3 ± 0.48 mm at $40 \mu\text{l}$, 16.8 ± 0.69 mm at $60 \mu\text{l}$, and 19.8 ± 0.20 mm at $80 \mu\text{l}$. Against *B. subtilis*, the extract showed activity only at $80 \mu\text{l}$, with a zone of inhibition of 8.90 ± 0.12 mm. The extract also exhibited activity against *Proteus* sp, with zones of inhibition of 8.90 ± 0.11 mm at $40 \mu\text{l}$ and 12.0 ± 0.00 mm at $80 \mu\text{l}$.

Notably, *P. aeruginosa* showed susceptibility to the extract, with zones of inhibition of 5.70 ± 0.54 mm at $20 \mu\text{l}$, 6.90 ± 0.25 mm at $40 \mu\text{l}$, 17.8 ± 0.25 mm at $60 \mu\text{l}$, and 13.5 ± 0.11 mm at $80 \mu\text{l}$ concentrations respectively (Table 5).

Table 5. Assessment of antibacterial potential of *C. asiaticum* bulb (KGVA 3) aqueous extract against clinically relevant bacteria

| Name of the bacteria | Zone of inhibition (mm) | | | |
|----------------------|-------------------------|------------------|------------------|------------------|
| | 20 μl | 40 μl | 60 μl | 80 μl |
| <i>B. subtilis</i> | - | - | - | 8.90 ± 0.12 |
| <i>E. coli</i> | 12.5 ± 0.36 | 15.3 ± 0.48 | 16.8 ± 0.69 | 19.8 ± 0.20 |
| <i>Proteus</i> sp | - | 8.90 ± 0.11 | - | 12.0 ± 0.00 |
| <i>P. aeruginosa</i> | 5.70 ± 0.54 | 6.90 ± 0.25 | 17.8 ± 0.25 | 13.5 ± 0.11 |

The values are expressed with Mean \pm Standard deviation

In the *C. asiaticum* bulb (KGVA 3) extract hydrated methanol extract were showed 9.60 ± 0.36 , 8.19 ± 0.00 and 9.16 ± 0.12 mm zone of inhibition against the *B. subtilis* at 40, 60 and $80 \mu\text{l}$ concentrations. The *E. coli* was observed with 5.30 ± 0.30 and 8.70 ± 0.00 mm zone of inhibitions at 40 and $80 \mu\text{l}$ concentration of *C. asiaticum* extract. Similarly, moderate growth

inhibitions observed from the *Proteus* sp. with 3.60 ± 0.10 and 10.0 ± 0.02 mm at 40 and 80 μ l concentration of *C. asiaticum* extract. In this regard, the maximum antifungal activity was observed from the *Pseudomonas aeruginosa* with 20.3 ± 0.45 mm zone of inhibition at 80 μ l concentration of *C. asiaticum* extract respectively (Table 6).

Table 6. Assessment of antibacterial potential of *C. asiaticum* bulb (KGVA 3) hydrated methanol extract against clinically relevant bacteria

| Name of the bacteria | Zone of inhibition (mm) | | | |
|----------------------|-------------------------|-----------------|-----------------|-----------------|
| | 20 μ l | 40 μ l | 60 μ l | 80 μ l |
| <i>B. subtilis</i> | - | 9.60 ± 0.36 | 8.19 ± 0.00 | 9.16 ± 0.12 |
| <i>E. coli</i> | - | 5.30 ± 0.30 | - | 8.70 ± 0.00 |
| <i>Proteus</i> sp | - | 3.60 ± 0.10 | - | 10.0 ± 0.02 |
| <i>P. aeruginosa</i> | - | - | - | 20.3 ± 0.45 |

The values are expressed with Mean \pm Standard deviation

Antifungal activity

Assessment of antifungal potential of *C. asiaticum* Bulb (KGVA 1) against clinically relevant fungi

In aqueous extract of *C. asiaticum* bulb (KGVA 1) extract showed following zone of inhibitions such as 11.2 ± 0.10 , 12.2 ± 0.10 and 14.1 ± 0.26 mm from *Aspergillus flavus* by 40, 60 and 80 μ l concentration. The 7.88 ± 0.30 mm growth inhibition was observed from the *A. fumigatus* at 80 μ l concentration of *C. asiaticum* bulb extract. *A. niger* was significantly inhibited with 12.1 ± 0.36 , 18.4 ± 0.20 , 20.4 ± 0.30 and 25.1 ± 0.15 mm zone of inhibition at 20, 40, 60 and 80 μ l concentration of *C. asiaticum* bulb extract. Remaining the *A. terreus* was not inhibited by *C. asiaticum* bulb extract (Table 7).

Table 7. Assessment of antifungal potential of *C. asiaticum* bulb (KGVA 1) aqueous extract against clinically relevant fungi

| Name of the fungi | Zone of inhibition (mm) | | | |
|---------------------|-------------------------|-----------------|-----------------|-----------------|
| | 20 μ l | 40 μ l | 60 μ l | 80 μ l |
| <i>A. flavus</i> | - | 11.2 ± 0.10 | 12.2 ± 0.10 | 14.1 ± 0.26 |
| <i>A. fumigatus</i> | - | - | - | 7.88 ± 0.30 |
| <i>A. niger</i> | 12.1 ± 0.36 | 18.4 ± 0.2 | 20.4 ± 0.30 | 25.1 ± 0.15 |
| <i>A. terreus</i> | - | - | - | - |

The values are expressed with Mean \pm Standard deviation

In hydrated methanol extract of *C. asiaticum* bulb (KGVA 1) extract was performed for the antifungal activity. The *Aspergillus flavus* was showed 5.81 ± 0.36 mm zone of inhibition at 80 μ l concentration. The 9.16 ± 0.00 , 12.6 ± 0.20 , 14.5 ± 0.36 and 14.6 ± 0.20 mm zone of inhibitions were observed from *A. fumigatus* at 20, 40, 60 and 80 μ l concentration of *C. asiaticum* bulb extract. The maximum growth inhibitions such as 9.33 ± 0.15 , 12.1 ± 0.78 , 13.5 ± 0.30 and 14.7 ± 0.30 mm were observed from *A. niger* at 20, 40, 60 and 80 μ l concentration of *C. asiaticum* bulb extract. There is no growth inhibitions were observed from the *A. terreus* (Table 8).

Table 8. Assessment of antifungal potential of *C. asiaticum* bulb (KGVA 1) hydrated methanol extract against clinically relevant fungi

| Name of the fungi | Zone of inhibition (mm) | | | |
|---------------------|-------------------------|-----------------|-----------------|-----------------|
| | 20 μ l | 40 μ l | 60 μ l | 80 μ l |
| <i>A. flavus</i> | - | - | - | 5.81 ± 0.36 |
| <i>A. fumigatus</i> | 9.16 ± 0.00 | 12.6 ± 0.20 | 14.5 ± 0.36 | 14.6 ± 0.20 |
| <i>A. niger</i> | 9.33 ± 0.15 | 12.1 ± 0.78 | 13.5 ± 0.30 | 14.7 ± 0.30 |
| <i>A. terreus</i> | - | - | - | 1.00 ± 0.00 |

Assessment of antifungal potential of *C. asiaticum* Bulb (KGVA 2) against clinically relevant fungi

An aqueous extract of *C. asiaticum* bulb (KGVA 2) extract showed 5.31 ± 0.00 mm zone of inhibition from *A. flavus* at 80 μ l concentration of *C. asiaticum* bulb extract. *A. niger* was significantly inhibited by 20, 40, 60 and 80 μ l concentration of *C. asiaticum* bulb extract with 8.61 ± 0.21 , 4.24 ± 0.11 , 9.61 ± 0.36 and 15.3 ± 1.48 mm zone of inhibition respectively. Remaining the *A. fumigatus* and *A. terreus* growth was not inhibited by at any concentrations of *C. asiaticum* bulb extract (Table 9).

Table 9. Assessment of antifungal potential of *C. asiaticum* bulb (KGVA 2) aqueous extract against clinically relevant fungi

| Name of the fungi | Zone of inhibition (mm) | | | |
|---------------------|-------------------------|-----------------|-----------------|-----------------|
| | 20 μ l | 40 μ l | 60 μ l | 80 μ l |
| <i>A. flavus</i> | - | - | - | 5.31 ± 0.00 |
| <i>A. fumigatus</i> | - | - | - | - |
| <i>A. niger</i> | 8.61 ± 0.21 | 4.24 ± 0.11 | 9.61 ± 0.36 | 15.3 ± 1.48 |
| <i>A. terreus</i> | - | - | - | - |

The values are expressed with Mean \pm Standard deviation

In the hydrated methanol extract of *C. asiaticum* bulb (KGVA 2) extract showed 5.81 ± 0.10 mm zone of inhibition against *A. flavus* at 80 μ l concentration. *A. fumigatus* growth was inhibited with 7.81 ± 0.20 mm by 80 μ l concentration of *C. asiaticum* bulb extract. In this investigation found that maximum antifungal activity from the *A. niger* with 3.25 ± 0.11 , 12.0 ± 0.33 and 12.6 ± 0.12 mm zone of inhibitions at 40, 60 and 80 μ l concentrations of *C. asiaticum* bulb extract. In hydrated methanol extract was also not effective for the *A. terreus* (Table 10).

Table 10. Assessment of antifungal potential of *C. asiaticum* bulb (KGVA 2) hydrated methanol extract against clinically relevant fungi

| Name of the fungi | Zone of inhibition (mm) | | | |
|---------------------|-------------------------|-----------------|-----------------|-----------------|
| | 20 μ l | 40 μ l | 60 μ l | 80 μ l |
| <i>A. flavus</i> | - | - | - | 5.81 ± 0.10 |
| <i>A. fumigatus</i> | - | - | - | 7.81 ± 0.20 |
| <i>A. niger</i> | - | 3.25 ± 0.11 | 12.0 ± 0.33 | 12.6 ± 0.12 |
| <i>A. terreus</i> | - | - | - | - |

The values are expressed with Mean \pm Standard deviation

Assessment of antifungal potential of *C. asiaticum* Bulb (KGVA 3) against clinically relevant fungi

In aqueous extract of *C. asiaticum* bulb (KGVA 3) extract showed 1.61 ± 0.30 mm zone of inhibition from *A. flavus* at 80 μ l concentration. The *A. fumigatus* was inhibited with 11.2 ± 0.31 mm at 80 μ l concentration of bulb extract. The 6.24 ± 0.11 and 7.31 ± 0.31 mm zone of inhibitions were observed from the 60 and 80 μ l concentration of *C. asiaticum* bulb extract against *A. niger* respectively. Moderately, *A. terreus* was inhibited with 21.1 ± 0.00 and 23.1 ± 0.01 mm zone of inhibition at 40 and 80 μ l *C. asiaticum* bulb extract (Table 11).

Table 11. Assessment of antifungal potential of *C. asiaticum* bulb (KGVA 3) aqueous extract against clinically relevant fungi

| Name of the fungi | Zone of inhibition (mm) | | | |
|---------------------|-------------------------|-----------------|-----------------|-----------------|
| | 20 μ l | 40 μ l | 60 μ l | 80 μ l |
| <i>A. flavus</i> | - | - | - | 1.61 ± 0.30 |
| <i>A. fumigatus</i> | - | - | - | 11.2 ± 0.31 |
| <i>A. niger</i> | - | - | 6.24 ± 0.11 | 7.31 ± 0.31 |
| <i>A. terreus</i> | - | 21.1 ± 0.00 | - | 23.1 ± 0.01 |

The values are expressed with Mean \pm Standard deviation

In hydrated methanol extract of *C. asiaticum* bulb (KGVA 3) were showed 2.14 ± 0.36 , 2.34 ± 0.26 , 3.64 ± 0.21 and 5.81 ± 0.31 mm zone of inhibitions at 20, 40, 60 and 80 μ l concentration. The maximum antifungal activity was observed against the *A. fumigatus* with following zone of inhibitions such as 12.5 ± 0.12 , 13.9 ± 0.54 , 14.5 ± 0.31 and 15.2 ± 0.00 mm at 20, 40, 60 and 80 μ l concentrations. The *A. niger* was moderately inhibited with following zone of inhibitions such as 8.61 ± 0.12 and 10.2 ± 0.31 mm at 20, 40, 60 and 80 μ l concentration of *C. asiaticum* bulb extract. There is no growth inhibition observed in the *A. terreus* respectively (Table 12).

Table 12. Assessment of antifungal potential of *C. asiaticum* bulb (KGVA 3) hydrated methanol extract against clinically relevant fungi

| Name of the fungi | Zone of inhibition (mm) | | | |
|---------------------|-------------------------|-----------------|-----------------|-----------------|
| | 20 μ l | 40 μ l | 60 μ l | 80 μ l |
| <i>A. flavus</i> | 2.14 ± 0.36 | 2.34 ± 0.26 | 3.64 ± 0.21 | 5.81 ± 0.31 |
| <i>A. fumigatus</i> | 12.5 ± 0.12 | 13.9 ± 0.54 | 14.5 ± 0.31 | 15.2 ± 0.00 |
| <i>A. niger</i> | 8.61 ± 0.12 | - | 10.2 ± 0.31 | - |
| <i>A. terreus</i> | - | - | - | - |

The values are expressed with Mean \pm Standard deviation

DISCUSSION

The phytochemical composition and antimicrobial activity of various plant extracts, including *C. asiaticum*, *C. martinii*, *C. tuberosa* and *T. indica*. The results of the qualitative phytochemical screening revealed the presence of various phytochemicals, including flavonoids, steroids, terpenoids, saponins, phenols, tannins and anthroquinones. Among these phytochemicals, steroids were found to be the most abundant followed by flavonoids (Purushotham *et al.*, 2019). The antimicrobial activity of *Crinum* species has been extensively studied, and the results have consistently shown that these plants possess broad-spectrum antimicrobial properties against various Gram-positive, Gram-negative bacteria and fungal pathogens (Minkah and Danguah, 2021; Hyun *et al.*, 2008; Goswami *et al.*, 2020; Fu *et al.*, 2020). The studies have demonstrated that the essential oil and extracts of *C. asiaticum* exhibit significant antifungal activities against pathogenic fungi including *Candida*

species (Naira *et al.*, 2017; Surain and Aneja, 2014; Noubissi *et al.*, 2019). The ethanolic extract of *C. asiaticum* has been shown to inhibit selected bacteria, while the dichloromethane extract has been found to be effective against oral and vaginal *Candida* species.

The antimicrobial activity of *C. jagus* has also been evaluated, and the results have shown that the extracts of this plant exhibit significant antimicrobial activity against clinically significant microorganisms (Udegbuma *et al.*, 2015; Azikiwe and Amazu, 2015). The water/ethanol extract of *C. jagus* has been found to be active against *Shigella flexneri*-induced diarrhea in rats, while the crude hydrated methanolic extract has been investigated for its effect on *Mycobacterium tuberculosis*. The crude alkaloid of *C. jagus* has been shown to inhibit Dengue virus infection, highlighting the potential of this plant as a source of antiviral agents (Akintola *et al.*, 2013). The biological effects of *Crinum macowanii* have also been studied, and the results have shown that this plant possesses antifungal, antiviral and antiplasmodial activities (Ka *et al.*, 2021; Maroyi, 2016). The aqueous fraction of *C. asiaticum* has been found to exhibit relatively higher antimicrobial potentials than the crude aqueous extract, indicating that serial extraction procedures can concentrate specific bioactive components. The ethyl acetate extract fraction has also been shown to exhibit antimicrobial activities, likely due to the presence of terpenoids, phenolics and flavonoids (Goswami *et al.*, 2020). The results of the current study are consistent with previous studies that have evaluated the antimicrobial activity of *Crinum* species (Kalid *et al.*, 2018; Rachdiati and Zakariya, 2018; Chanda and Baravalia, 2010).

The hydrated methanolic extracts of *C. asiaticum* have been shown to exhibit significant antimicrobial activity against *S. flexneri* and *Proteus* sp, while the ethanol extracts have been found to inhibit *T. viride* and *Cladosporium* sp. The antimicrobial activity and chemical constituents of plants have been confirmed in different studies, highlighting the potential of *Crinum* species as a source of natural antimicrobial

agents (Emad *et al.*, 2009; Sunilson *et al.*, 2009; Divya *et al.*, 2014; Lewis and Ausubel, 2006; Ernst, 2005; Ahmad *et al.*, 2008; Janovska *et al.*, 20003; Mahata and Sharma, 2018).

The use of *Crinum* species as a source of antimicrobial agents has several advantages, including their broad-spectrum activity, low toxicity, and potential for use in combination with conventional antimicrobial agents. The ethanol extract of *C. asiaticum* also showed significant antimicrobial activity against *T. viride*, with an inhibition zone of 12 ± 0.87 mm.

The hydrated methanol extract of *C. martinii* showed similar inhibition zones against *T. viride* and *Cladosporium* sp., with values of 8 ± 0.27 mm and 8 ± 0.21 mm, respectively. The partial inhibitory effect of the hydrated methanolic and ethanolic extracts of *C. tuberosa* and *T. indica* against the test microorganisms suggests that these plants may also possess antimicrobial properties (Purushotham *et al.*, 2019). The results of the current study demonstrate the antibacterial potential of *C. asiaticum* bulb (KGVA 1) extracts against a range of clinically relevant bacteria.

The hydrated methanol extract showed significant activity against *E. coli*, *B. subtilis*, *Proteus* sp, and *P. aeruginosa*, while the aqueous extract showed activity against *E. coli*, *B. subtilis*, *Proteus* sp, and *P. aeruginosa*. These findings suggest that *C. asiaticum* bulb extracts may be a useful natural source of antibacterial agents. The results of this study demonstrate the antibacterial potential of *C. asiaticum* bulb (KGVA 2) extracts against a range of clinically relevant bacteria. The hydrated methanol extract showed significant activity against *E. coli*, *Proteus* sp and *P. aeruginosa*, while the aqueous extract showed activity against *Proteus* sp.

These findings suggest that *C. asiaticum* bulb extracts may be a useful natural source of antibacterial agents, particularly against *P. aeruginosa*.

The results of the present study demonstrated the antibacterial potential of *C. asiaticum* bulb (KGVA 3) extracts against a range of clinically relevant bacteria. The hydrated methanol extract showed significant activity against *P. aeruginosa* followed by *Proteus* sp., *B. subtilis* and *E. coli*, while the aqueous extract showed activity against *E. coli* and *P. aeruginosa*.

These findings suggested that *C. asiaticum* bulb extracts may be a useful natural source of antibacterial agents. The results of this study demonstrated the antifungal potential of *C. asiaticum* bulb (KGVA 1) extracts against a range of clinically relevant fungi. The hydrated methanol extract showed significant activity against *A. niger* followed by *A. fumigatus* and *A. flavus*, while the aqueous extract showed activity against *A. niger* and *A. flavus*. These findings suggest that *C. asiaticum* bulb extracts may be a useful natural source of antifungal agents particularly against *A. niger* and *A. fumigatus*. The results of this study demonstrated the antifungal potential of *C. asiaticum* bulb (KGVA 2) extracts against a range of clinically relevant fungi. The hydrated methanol extract showed significant activity against *A. niger* and *A. fumigatus*, while the aqueous extract showed activity against *A. niger* and *A. flavus*. These findings suggested that *C. asiaticum* bulb extracts may be a useful natural source of antifungal agents, particularly against *A. niger*. The results of this study demonstrate the antifungal potential of *C. asiaticum* bulb (KGVA 3) extracts against a range of clinically relevant fungi. The hydrated methanol extract showed significant activity against *A. fumigatus* and *A. niger*, while the aqueous extract showed activity against *A. terreus* and *A. fumigatus*. These findings suggested that *C. asiaticum* bulb extracts may be a useful natural source of antifungal agents particularly against *A. fumigatus* and *A. terreus*.

CONCLUSION

This comprehensive study highlights the promising antimicrobial potential of *C. asiaticum* bulb extracts from three different areas (KGVA 1, KGVA 2 and

KGVA 3) against a range of clinically relevant bacteria and fungi. The findings demonstrate that both aqueous and hydrated methanol extracts exhibit broad-spectrum antibacterial and antifungal activities, with varying degrees of efficacy against different microorganisms. Notably, the hydrated methanol extract of KGVA 2 showed remarkable antibacterial activity against *Pseudomonas aeruginosa*, while the aqueous extract of KGVA 3 displayed significant antifungal activity against *Aspergillus* species. These results suggest that *C. asiaticum* bulb extracts may be a valuable source for the development of novel antimicrobial agents, warranting further investigation into their bioactive compounds and mechanisms of action. Future studies should focus on isolating and characterizing the active compounds responsible for these antimicrobial effects, as well as evaluating their potential applications in pharmaceutical and agricultural settings.

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