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Screening of an medicinal plant *Crinum asiaticum* L. bulb extracts for their proximate composition, phytochemical analysis and antioxidant activity

K. Gowthaman[*1], P. Prakash[1], V. Ambikapathy[1], S. Babu[1], A. Panneerselvam[2]

[1]*PG and Department of Botany, A.V.V.M Sri Pushpam College (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli- 24), Poondi, Thanjavur, Tamil Nadu, India*

[2]*Indian Biotrack Research Institute, Thanjavur, Tamil Nadu, India*

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ABSTRACT

Crinum asiaticum, a traditional medicinal plant has been used for various therapeutic purposes. Therefore, the present study aims to investigate the proximate composition, phytochemical analysis and antioxidant activities of *C. asiaticum* bulb extracts. *C. asiaticum* bulb were collected from three different district of Thanjavur, Nagappattinam, Namakkal and analyzed for proximate composition and phytochemical analysis. The results showed that *C. asiaticum* bulb extracts contained various bioactive compounds including alkaloids, glycosides, flavonoids and phenolic acids. KGVA extracts (KGVA 1, KGVA 2 and KGVA 3) were evaluated for antioxidant activity using H_2O_2, reducing power and DPPH assay. KGVA 1 and KGVA 2 showed significant antioxidant activity, comparable to ascorbic acid and Vitamin C. KGVA 1 had the highest activity in the DPPH assay. The extracts also contained various polyphenolic compounds including neochlorogenic acid, protocatechuic acid and vanillic acid. This study demonstrates the potential of *C. asiaticum* bulb extracts as a rich source of bioactive compounds. The results suggested that *C. asiaticum* may be a valuable plant for the development of new therapeutic agents particularly for the treatment of breast cancer and microbial infections.

***Corresponding Author:** K. Gowthaman ✉ gautham29480@gmail.com

* https://orcid.org/0009-0007-1368-9432

Co-authors:
Ambikapathy: https://orcid.org/0000-0002-0902-301X
Babu: https://orcid.org/0000-0003-1473-4796
Panneerselvam: https://orcid.org/0000-0001-6488-6523

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, 2018).

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). The family is characterized by its unique isoquinoline alkaloids which exhibit a wide range of biological activities including potential therapeutic properties. These alkaloids have been extensively studied for their medicinal value, highlighting the significance of the Amaryllidaceae family in the development of new pharmaceuticals (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). Spanning across continents, including Asia, Africa, America and Australia, the Crinum genus exhibits a remarkable diversity of species. In India, specifically, the genus is represented by a notable 15 species, each with unique characteristics and classifications. This introduction sets the stage for exploring the complexities and significance of the Crinum genus, with a focus on its Indian species (Lekhak and Yadav, 2012).

The Crinum genus, comprising both terrestrial and aquatic species, has been found to possess a wide range of medicinal properties including antioxidant, antimicrobial and anti-inflammatory effects. These therapeutic benefits are attributed to the presence of bioactive alkaloids which have been shown to exhibit antimalarial, antiviral, anti-tumor and anti-diabetic activities (Jagtap *et al.*, 2014). Despite their potential, the phytochemical properties of Indian Crinum species remain understudied, with limited research focused on their alkaloid content (Swati *et al.*, 2021). Furthermore, the medicinal potential of many Indian *Crinum* species remains untapped highlighting the need for further investigation into the therapeutic applications of these plants. Therefore, the present study focused the Indian Crinum asiaticum plants for the therapeutically applications.

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 nutritional composition of *C. americanum* has been found to be rich and diverse, providing valuable insights into its potential as a functional food or medicinal plant. Analysis of its nutritional content has revealed significant levels of moisture, ash, protein, lipids, dietary fiber and various carbohydrates, including total sugar, reducing and non-reducing sugars. These findings suggest that *C. americanum* may be a valuable source of essential nutrients and bioactive compounds, warranting further investigation into its potential

health benefits and therapeutic applications (Renata *et al.*, 2023).

The bulb of *C. asiaticum*, a traditional medicinal plant, has been found to be a treasure trove of phytochemicals, possessing a unique combination of bioactive compounds with potential therapeutic properties. Rich in alkaloids, glycosides, flavonoids, phenolic acids and terpenoids, the bulb of *C. asiaticum* have been shown to exhibit a range of biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects. The phytochemical profile of *C. asiaticum* bulb has sparked significant interest in the scientific community at unlocking their full therapeutic potential and exploring their potential applications in the prevention and treatment of various diseases (Maroyi, 2016).

Crinum asiaticum exhibited the significant antioxidant activities which have been evaluated through various in-vitro assays including H_2O_2 scavenging assay, reducing power assay and DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay. Most of the studies were demonstrated the ability of *C. asiaticum* extracts to scavenge free radicals, reduce oxidative stress and protect against cellular damage, highlighting its potential as a natural antioxidant agent (Soni *et al.*, 2015).

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 and authenticity of plant material

Crinum asiaticum bulb (KGVA 1, KGVA 2 and KGVA 3) samples were sourced from three different places of Thanjavur, Nagappattinam and Namakkal, Tamil Nadu, India. The plant specimens were subsequently identified and authenticated by experts. A voucher specimen, designated as SPCH-NT-072–74, SPCH-NT-072-75 and SPCH-NT-072-76 were deposited in the department's herbarium for future references (Jayakumar, 2015).

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 four distinct solvents of aqueous, benzene, hexane and 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).

Proximate composition

The proximate composition of C.asiaticum bulb (KGVA 1, KGVA 2 and KGVA 3) were determined with parameters such as ash, soluble ash, crude fiber, moisture, protein, carbohydrate and lipid content were analyzed. These analyses were conducted using standardized methods outlined by the American Association of Cereal Chemists (AACC, 2000).

In-vitro preliminary phytochemical analysis

An *in-vitro* phytochemical of *C. asiaticum* bulb with different solvents such as aqueous, benzene, hexane and methanol extracts were performed and estimated various phytochemical compounds including alkaloids, amino acids, coumarins, flavonoids, glycosides, phenols, quinones, saponins, steroids and terpenoids (Harborne, 1998).

In-vitro antioxidant assays

H_2O_2 assay

The hydrogen peroxide (H_2O_2) scavenging ability of the *C. asiaticum* bulb (KGVA 1, KGVA 2 and KGVA 3) extract was determined using a method previously

described method (Ruch *et al.*, 1989). Aliquots of 0.1 mL of the extract, at concentrations ranging from 25-400µg/mL, were transferred into eppendorf tubes, and the volume was adjusted to 0.4 mL with 50 mM phosphate buffer (pH 7.4). Subsequently, 0.6 mL of a 2 mM H_2O_2 solution was added to the mixture. The reaction mixture was vortexed and after a 10-minute reaction period, the absorbance was measured at 230 nm. Ascorbic acid served as the positive control. The H_2O_2 scavenging ability of the extracts was calculated using a specific equation.

H_2O_2 scavenging activity (%)= [(A0-A1)/A0]×100

Reducing power assay

The reducing power of *C. asiaticum* bulb extracts (KGVA 1, KGVA 2 and KGVA 3) was assessed by mixed with 1mL of each extract at various concentrations (100, 200, 300, 400 and 500µg/mL) with 2.5mL of phosphate buffer (0.2M, pH 6.6) and 2.5mL of 1% potassium ferricyanide solution were added. The resulting mixture was then incubated at 50°C for 20 minutes. The incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture, which was subsequently centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge. The supernatant (2 mL) was then mixed with an equal volume of distilled water and 0.5 mL of 0.1% ferric chloride solution. The absorbance of solution was measured at 700 nm using a UV-Visible spectrophotometer. Each experiment was conducted in triplicate at each concentration to ensure reliable results (Oyaizu, 1986).

DPPH assay

The antioxidant activity of *C. asiaticum* bulb extracts (KGVA 1, KGVA 2 and KGVA 3) with different concentrations of 100, 200, 300, 400 and 500 µg/mL was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, where ascorbic acid solutions served as standard. The DPPH powder was dissolved in methanol (p.a.) to prepare a test solution, which was then combined with 2 mL of the sample solution in a test tube. The mixture was vortexed until homogeneous and incubated in the dark for 30 minutes. The absorbance was measured at 517 nm using a visible spectrophotometer. The percentage of inhibition was calculated using a specific formula (Bhalodia *et al.*, 2013).

Activity (%)= [(A-B)/A]×100

RESULTS

Proximate composition and nutrient content of *Crinum asiaticum* bulb from three different places

The proximate composition and nutrient content of *C. asiaticum* bulb from three different places (KGVA 1, KGVA 2 and KGVA 3) were analyzed. The ash content of the bulb ranged from 1.00 to 1.11mg/10g, with KGVA 1 having the highest ash content. The soluble ash content was similar across all three locations, ranging from 1.08 to 1.13mg/10g. The crude fiber content was highest in KGVA 1 (2.11µg/10g) and lowest in KGVA 2 and KGVA 3 (1.08 and 1.09µg/10g) respectively. The moisture content was highest in KGVA 1 (7.06%) and lowest in KGVA 2 and KGVA 3 (5.06 and 5.16%). The protein content was highest in KGVA 1 (2.04µg/10g) and lowest in KGVA 2 and KGVA 3 (1.14µg/10g). The carbohydrate content was similar across all three locations, ranging from 2.00 to 2.07µg/10g. The lipid content was similar across all three locations, ranging from 0.98 to 1.11µg/10g (Fig. 1).

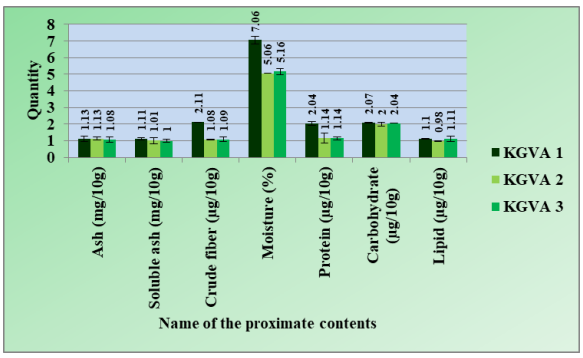

Fig. 1. Proximate composition of *Crinum asiaticum* bulb of three different places

Phytochemical analysis

The phytochemical analysis of *C. asiaticum* bulb of three different species (KGVA 1, KGVA 2 and KGVA 3) revealed that the presence of various bioactive compounds including alkaloids, glycosides, flavonoids and phenolic acids. The quantitative analysis showed significant amounts of these compounds detected.

These findings suggest that *C. asiaticum* bulb is a rich source of phytochemicals with potential medicinal properties.

Qualitative phytochemical analysis

The qualitative phytochemical analysis of *C. asiaticum* bulb was performed on three different samples (KGVA 1, KGVA 2 and KGVA 3) using four different solvents (aqueous, benzene, hexane and methanol). The results showed that the alkaloids, flavonoids, phenols, steroids and terpenoids were present in all three samples and in all four solvents. Amino acids were present in KGVA 1 and KGVA 3 in all solvents, but absent in KGVA 2 in benzene and methanol solvents. Coumarins were present in KGVA 1 and KGVA 3, but absent in KGVA 2 in aqueous and hexane solvents. Glycosides were present in KGVA 1 and KGVA 2, but absent in KGVA 3 in hexane solvent. Quinones were present in KGVA 1 and KGVA 2, but absent in KGVA 3 in aqueous and methanol solvents. Saponins were present in KGVA 2 but absent in KGVA 3 and KGVA 1 (Table 1a-c).

Table 1a. Qualitative phytochemical analysis of *C. asiaticum* bulb (KGVA 1)

Phytochemical compounds	Different solvents			
	Aqueous	Benzene	Hexane	Methanol
Alkaloids	+	+	+	+
Amino acids	+	+	+	+
Coumarins	+	-	+	-
Flavonoids	+	+	+	+
Glycoside	+	+	-	+
Phenols	+	+	+	+
Quinones	+	-	-	-
Saponin	-	-	-	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+

(+)Present, (-) Absent

Table 1b. Qualitative phytochemical analysis of *C. asiaticum* bulb (KGVA 2)

Phytochemical compounds	Different solvents			
	Aqueous	Benzene	Hexane	Methanol
Alkaloids	+	+	+	+
Amino acids	+	-	+	-
Coumarins	-	-	-	+
Flavonoids	+	+	+	+
Glycoside	+	+	+	+
Phenols	+	-	-	+
Quinones	+	+	-	-
Saponin	+	+	+	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+

(+) Present, (-) Absent

Table 1c. Qualitative phytochemical analysis of *C. asiaticum* bulb (KGVA 3)

Phytochemical compounds	Different solvents			
	Aqueous	Benzene	Hexane	Methanol
Alkaloids	+	+	+	+
Amino acids	+	+	+	+
Coumarins	+	-	-	-
Flavonoids	+	-	+	+
Glycoside	-	+	-	-
Phenols	+	+	+	+
Quinones	-	+	+	-
Saponin	-	-	-	+
Steroids	+	+	+	+
Terpenoids	+	+	+	+

(+) Present, (-) Absent

Quantitative phytochemical analysis

Quantitative phytochemical analysis of C. asiaticum bulb (KGVA 1)

The quantitative phytochemical analysis of *C. asiaticum* bulb (KGVA 1) revealed that the presence of various bioactive compounds in different solvents. Alkaloids were present in all four solvents, with the highest quantity (2.91±0.00µg/10g) in methanol and the lowest (2.05±0.00µg/10g) in benzene. Amino acids were also present in all four solvents, with the highest quantity (2.70±0.06µg/10g) in methanol and the lowest (1.32±0.26µg/10g) in benzene. Coumarins were present in aqueous and hexane solvents, with quantities of 2.84±0.18µg/10g and 1.30±0.06µg/10g respectively. Flavonoids were present in all four solvents, with the highest quantity (1.65±0.26µg/10g) in hexane and the lowest (1.00±0.00µg/10g) in benzene. Glycosides were present in aqueous, benzene, and methanol solvents, with quantities of 1.02±0.06µg/10g, 1.09±0.69µg/10g and 1.56±0.00µg/10g, respectively. Phenols were present in all four solvents, with the highest quantity (2.00±0.33µg/10g) in methanol and the lowest (1.33±0.12µg/10g) in hexane. Quinones were present only in aqueous solvent with a quantity of 1.07±0.15µg/10g. Saponins were present only in methanol solvent with a quantity of 2.33±0.12µg/10g. Steroids were present in all four solvents with the highest quantity (2.13±0.16µg/10g) in methanol and the lowest (1.30±0.20µg/10g) in benzene. Terpenoids were present in all four solvents, with the highest quantity (1.78±0.00µg/10g) in aqueous and the lowest (1.32±0.03µg/10g) in methanol.

Overall, the results suggest that *C. asiaticum* bulb (KGVA 1) is a rich source of various bioactive compounds, with methanol being the most effective solvent for extracting these compounds (Table 2).

Table 2. Quantitative phytochemical analysis of *C. asiaticum* bulb (KGVA 1)

Phytochemical compounds	Quantity (µg/10g)			
	Aqueous	Benzene	Hexane	Methanol
Alkaloids	2.70±0.27	2.05±0.00	2.15±0.36	2.91±0.00
Amino acids	2.30±0.23	1.32±0.26	1.63±0.12	2.70±0.06
Coumarins	2.84±0.18	-	1.30±0.06	-
Flavonoids	1.25±0.30	1.00±0.00	1.65±0.26	1.13±0.02
Glycoside	1.02±0.06	1.09±0.69	-	1.56±0.00
Phenols	1.96±0.34	1.75±0.22	1.33±0.12	2.00±0.33
Quinones	1.07±0.15	-	-	-
Saponin	-	-	-	2.33±0.12
Steroids	1.59±0.30	1.30±0.20	1.63±0.25	2.13±0.16
Terpenoids	1.78±0.00	1.63±0.00	1.42±0.12	1.32±0.03

Mean ± Standard deviation

Quantitative phytochemical analysis of C. asiaticum bulb (KGVA 2)

The quantitative phytochemical analysis of *C. asiaticum* bulb (KGVA 2) revealed that the presence of various bioactive compounds in different solvents. Alkaloids were present in all four solvents, with the highest quantity (8.22±0.57µg/10g) in benzene and the lowest (7.16±0.01µg/10g) in hexane. Amino acids were present in aqueous and hexane solvents, with quantities of 3.00±0.00µg/10g and 2.36 ± 0.30µg/10g, respectively. Coumarins were present only in methanol solvent, with a quantity of 1.02±0.31µg/10g. Flavonoids were present in aqueous, benzene and methanol solvents, with quantities of 2.25±0.36µg/10g, 1.69±0.26µg/10g and 2.27±0.93µg/10g respectively. Glycosides were present in all four solvents, with the highest quantity (1.25±0.06µg/10g) in hexane and the lowest (1.03±0.39µg/10g) in benzene. Phenols were present in aqueous, hexane and methanol solvents with quantities of 1.02±0.13µg/10g, 1.17±0.38µg/10g and 1.31±0.00µg/10g respectively. Quinones were present in aqueous and benzene solvents, with quantities of 1.36±0.36µg/10g and 1.00±0.06µg/10g respectively. Saponins were present in all four solvents, with the highest quantity

(3.47±0.01µg/10g) in methanol and the lowest (1.74±0.00µg/10g) in benzene. Steroids were present in all four solvents, with the highest quantity (1.58±0.04µg/10g) in benzene and the lowest (1.13±0.08µg/10g) in hexane. Terpenoids were present in all four solvents, with the highest quantity (8.22±0.57µg/10g) in benzene and the lowest (7.16±0.11µg/10g) in hexane. Overall, the results suggest that *C. asiaticum* bulb (KGVA 2) is a rich source of various bioactive compounds, with benzene being the most effective solvent for extracting alkaloids, terpenoids and steroids and methanol being the most effective solvent for extracting saponins (Table 3).

Table 3. Quantitative phytochemical analysis of *C. asiaticum* bulb (KGVA 2)

Phytochemical compounds	Quantity (µg/10g)			
	Aqueous	Benzene	Hexane	Methanol
Alkaloids	7.74±0.18	8.22±0.57	7.16±0.01	8.02±0.02
Amino acids	3.00±0.00	-	2.36±0.30	-
Coumarins	-	-	-	1.02±0.31
Flavonoids	2.25±0.36	1.69±0.26	-	2.27±0.93
Glycoside	1.24±0.86	1.03±0.39	1.25±0.06	1.06±0.08
Phenols	1.02±0.13	-	1.17±0.38	1.31±0.00
Quinones	1.36±0.36	1.00±0.06	-	-
Saponin	3.15±0.23	1.74±0.00	2.27±0.11	3.47±0.01
Steroids	1.22±0.04	1.58±0.04	1.13±0.08	1.16±0.04
Terpenoids	7.74±0.18	8.22±0.57	7.16±0.11	8.02±0.02

Mean ± Standard deviation

Table 4. Quantitative phytochemical analysis of *C. asiaticum* bulb (KGVA 3)

Phytochemical compounds	Quantity (µg/10g)			
	Aqueous	Benzene	Hexane	Methanol
Alkaloids	3.00±0.33	2.36±0.00	2.10±0.33	2.63±0.33
Amino acids	1.39±0.26	1.22±0.02	1.20±0.14	1.30±0.00
Coumarins	1.59±0.37	-	-	-
Flavonoids	1.66±0.15	-	1.69±0.22	1.63±0.22
Glycoside	-	1.36±0.22	-	-
Phenols	1.69±0.52	1.47±0.69	1.22±0.45	1.20±0.12
Quinones	-	0.98±0.32	1.01±0.52	-
Saponin	-	-	-	1.00±0.00
Steroids	1.47±0.60	1.21±0.28	1.31±0.21	1.37±0.81
Terpenoids	1.58±0.36	1.20±0.36	1.88±0.22	1.45±0.68

Mean ± Standard deviation

Quantitative phytochemical analysis of C. asiaticum bulb (KGVA 3)

The quantitative phytochemical analysis of *C. asiaticum* bulb (KGVA 3) revealed that the presence of various bioactive compounds in different solvents. Alkaloids were present in all four solvents, with the highest quantity (3.00±0.33µg/10g) in aqueous and the lowest (2.10±0.33µg/10g) in hexane. Amino acids were present in all four solvents, with the highest quantity (1.39±0.26µg/10g) in aqueous and the lowest (1.20±0.14µg/10g) in hexane. Coumarins were present only in aqueous solvent, with a quantity of 1.59±0.37µg/10g. Flavonoids were present in aqueous, hexane, and methanol solvents, with quantities of 1.66±0.15µg/10g, 1.69±0.22µg/10g and 1.63±0.22µg/10g respectively. Glycosides were present only in benzene solvent, with a quantity of 1.36±0.22µg/10g. Phenols were present in all four solvents, with the highest quantity (1.69±0.52µg/10g) in aqueous and the lowest (1.20±0.12µg/10g) in methanol. Quinones were present in benzene and hexane solvents, with quantities of 0.98±0.32µg/10g and 1.01±0.52µg/10g, respectively. Saponins were present only in methanol solvent, with a quantity of 1.00±0.00µg/10g. Steroids were present in all four solvents, with the highest quantity (1.47±0.60µg/10g) in aqueous and the lowest (1.21±0.28µg/10g) in benzene. Terpenoids were present in all four solvents, with the highest quantity (1.88±0.22µg/10g) in hexane and the lowest (1.20±0.36µg/10g) in benzene.

Overall, the results suggest that *C. asiaticum* bulb (KGVA 3) is a maximum source of various bioactive compounds with aqueous solvent being the most effective for extracting alkaloids, amino acids and phenols and hexane solvent being the most effective for extracting terpenoids (Table 4).

Antioxidant activity

H_2O_2 assay

The antioxidant activity of the bulb extracts (KGVA 1, KGVA 2 and KGVA 3) was evaluated using the H2O2 assay. The table showed the percentage inhibition of H2O2 by different concentrations of ascorbic acid and the extracts. The IC50 values, which represented the concentration of the extract required to inhibit 50% of H2O2, were calculated and are presented. The lower the IC50 value, the higher the antioxidant activity of the extract.

Ascorbic acid, a known antioxidant, exhibited a dose-dependent antioxidant activity with an IC50 value of 18.2µg/ml. KGVA 1 and KGVA 2 extract showed similar antioxidant activity to ascorbic acid, with IC50 values of 18.1 and 18.2µg/ml, respectively. KGVA 3 extract showed lower antioxidant activity, with an IC50 value of 38.5µg/ml. The antioxidant activity of the extracts decreased in the order of KGVA 1 > KGVA 2 > KGVA 3. Overall, the results suggest that the bulb extracts of C.asiaticum, particularly KGVA 1 and KGVA 2, possess significant antioxidant activity which may contribute to their potential health benefits (Fig. 2).

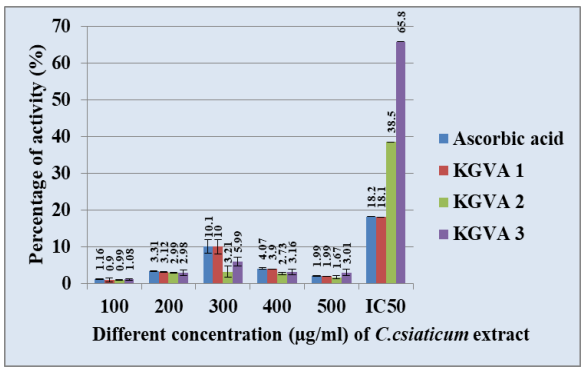

Fig. 2. *In-Vitro* H_2O_2 Antioxidant activity from different samples of *C. asiaticum* Bulb methanol extract

Reducing power assay

The reducing power of the bulb extracts (KGVA 1, KGVA 2 and KGVA 3) was evaluated using the reducing power assay. The table shows the absorbance values at different concentrations of ascorbic acid and the extracts. The IC50 values, which represent the concentration of the extract required to achieve 50% of the maximum reducing power were calculated. The lower the IC50 value, the higher the reducing power of the extract. Ascorbic acid, a known antioxidant, exhibited a dose-dependent reducing power, with an IC50 value of 53.5µg/ml. KGVA 1 and KGVA 2 extracts showed similar reducing power to ascorbic acid, with IC50 values of 52.9 and 53.8µg/ml respectively. The KGVA 3 extract showed lower reducing power, with an IC50 value of 79.2µg/ml. The reducing power of the extracts decreased in the order of KGVA 1 ≈ KGVA 2 > Ascorbic acid > KGVA 3. Overall, the results suggested that the extracts, particularly KGVA 1 and KGVA 2, possess significant reducing power, which may contribute to their potential antioxidant and health benefits. The reducing power of the extracts is likely due to the presence of bioactive compounds that can donate electrons and reduce oxidative stress (Fig. 3).

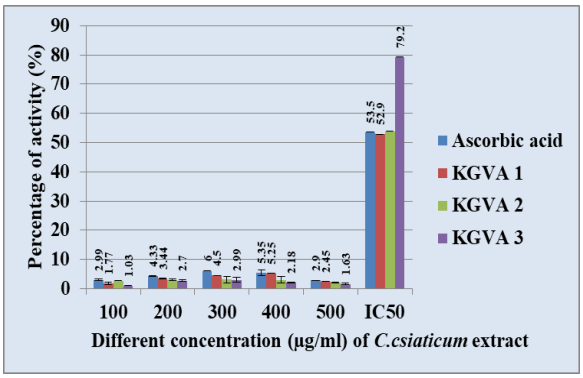

Fig. 3. *In-Vitro* reducing power antioxidant activity from different samples of *C. asiaticum* Bulb methanol extract

DPPH assay

The antioxidant activity of the bulb extracts (KGVA 1, KGVA 2 and KGVA 3) was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The table shows the percentage inhibition of DPPH by different concentrations of Vitamin C and the extracts.

The IC50 values, which represent the concentration of the extract required to inhibit 50% of DPPH, were calculated and are presented in the table. The lower the IC50 value, the higher the antioxidant activity of the extract. Vitamin C, a known antioxidant, exhibited a dose-dependent antioxidant activity, with an IC50 value of 34.3µg/ml. KGVA 1 extract showed the highest antioxidant activity, with an IC50 value of 28.8µg/ml, which is lower than that of Vitamin C. KGVA 2 extract showed similar antioxidant activity to Vitamin C, with an IC50 value of 34.3µg/ml. The KGVA 3 extract showed slightly lower antioxidant activity, with an IC50 value of 35.2µg/ml. The antioxidant activity of the extracts decreased in the order of KGVA 1 > KGVA 2 ≈ Vitamin C > KGVA 3. Overall, the results suggested that the bulb extracts, particularly KGVA 1, possess significant antioxidant activity which may contribute to their potential health benefits. The antioxidant activity of the extracts is likely due to the presence of bioactive compounds that can scavenge free radicals and reduce oxidative stress (Fig. 4).

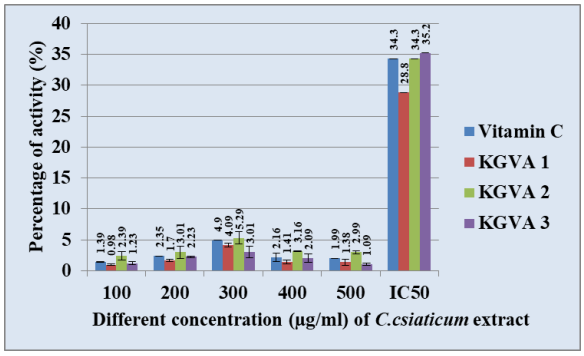

Fig. 4. *In-Vitro* DPPH Antioxidant activity from different samples of *C. asiaticum* Bulb methanol extract

DISCUSSION

The results of the studies on *Crinum americanum* L. and its bulb provide valuable insights into the nutritional and phytochemical profiles of this plant. The study on *C. americanum* L. found that the plant has a rich nutritional profile with high antioxidant capacity, total phenolic compounds and total alkaloids. The optimized solvent system used in the study resulted in the identification of 89 bioactive compounds including alkaloids, phenolic compounds and fatty acids. His study also found that the extracts did not show ex-vivo cytotoxicity against human peripheral blood mononuclear cells, suggesting that the plant was safe for consumption (Renata *et al.*, 2023). The present study on *C. asiaticum* bulb found that the proximate composition and nutrient content varied slightly across different locations, but the overall nutrient profile was similar. This suggested that the bulb from different locations have similar nutritional value. The results of this study provide valuable information on the nutritional content of *C. asiaticum* bulb, which can be useful for food and pharmaceutical applications.

The studies highlighted the potential of Crinum species as unconventional edible plants (UEPs) with medicinal value. The use of *C. americanum* L. in traditional medicine in coastal communities in Brazil to treat cancer suggests that the plant has been recognized for its therapeutic properties (Silva *et al.*, 2020). The determination of the nutritional profile and inorganic components of these plants and their extracts is of great interest for public wellbeing, as certain elements and nutrients play important roles in human and animal health.

The comparison of the results between the both studies suggests that Crinum species may have similar nutritional and phytochemical profiles, despite some variations in the proximate composition and nutrient content. The identification of bioactive compounds in *C. americanum* and the antioxidant capacity of *C. asiaticum* bulb suggest that these plants may have potential health benefits including anti-inflammatory, antimicrobial and anticancer properties (Miliao *et al.*, 2022; Castroalba *et al.*, 2019). Overall, the results of these studies contributed to the understanding of the nutritional and phytochemical profiles of Crinum species and highlighted their potential as UEPs with medicinal values. Further research is needed to fully explore the therapeutic properties of these plants and to determine their safety and efficacy for human consumption. The present study analyzed the

proximate composition and nutrient content of *C. asiaticum* bulb from three different locations, providing valuable insights into the nutritional profile of this plant. The results showed variations in the ash, crude fiber, moisture and protein content among the three locations, with KGVA 1 having the highest values for these parameters. In contrast, the soluble ash, carbohydrate and lipid content were relatively consistent across the three locations. The observed variations in nutrient content among the different locations may be attributed to factors such as soil type, climate and agricultural practices. The higher ash and crude fiber content in KGVA 1 bulb suggested that they may have been grown in soil with higher mineral content or exposed to more favorable environmental conditions.

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 present study of phytochemical composition of the three samples showed some variations. KGVA 1 and KGVA 2 had a similar phytochemical profile, while KGVA 3 had a slightly different profile. The qualitative phytochemical analysis of *C. asiaticum* bulb revealed the presence of a range of bioactive compounds, including alkaloids, flavonoids, phenols, steroids and terpenoids. The results suggested that the phytochemical composition of *C. asiaticum* bulb can vary depending on the sample and solvent system used. Further studies were needed to fully characterize the phytochemical composition of *C. asiaticum* bulb and to explore its potential medicinal properties.

The antioxidant capacity of extracts from the leaves, bulb and roots of *C. americanum* L. was evaluated using DPPH and FRAP assays, as well as total phenolic content (TPC) and total alkaloid content (TAC) as screening methods. The results showed that the DPPH levels of the leaf, bulb and root extracts were higher than those reported by Gomes *et al.* (2022), indicating a stronger antioxidant capacity. The presence of phytochemicals such as phenolic compounds, flavonoids, tannins, alkaloids, vitamins and carotenoids is known to contribute to antioxidant activity (Patel *et al.*, 2018; Ghane *et al.*, 2018; Attar and Ghane, 2019; Patel and Ghane, 2021; Patel *et al.*, 2020). The antioxidant potential of *C. americanum* L. extracts is consistent with previous studies on other *Crinum* species, which have demonstrated significant antioxidant activity. For example, the ethanolic extract of *C. asiaticum* has been shown to have protective effects on human erythrocytes (Ilavenil *et al.*, 2011), while the bulb of *C. asiaticum* have exhibited remarkable free radical scavenging ability (Uddin *et al.*, 2015). The antioxidant activity of *C. asiaticum* leaves has also been demonstrated in alloxan-induced diabetic rats (Indradevi *et al.*, 2015), and the methanolic extract of *C. asiaticum* has shown antioxidant effects (Ghane *et al.*, 2018). Similarly, the aqueous leaf extract of *C. asiaticum* has been found to have potent DPPH radical scavenging activity (Ofori *et al.*, 2021). The antioxidant activity of Crinum species is not limited to *C. asiaticum*. For example, the leaves and bulb of *C. jagus* have been found to be important sources of antioxidant compounds (Alawode *et al.*, 2019), and the methanolic bulb extract of *C. bulbispermum* has shown mild radical scavenging activity (Adewusi and Steenkamp, 2011). The leaf extracts of *C. bulbispermum* have also been found to have modest antioxidant activity in a thiobarbituric acid reactive substances assay (Yui *et al.*, 1998). The present study of H_2O_2 assay measured the ability of the extracts to scavenge hydrogen peroxide, a reactive oxygen species (ROS) that can cause oxidative damage to cells. The results showed that KGVA 1 and KGVA 2 extracts exhibited similar antioxidant activity to ascorbic acid, a known antioxidant, with IC50 values of 18.1 and 18.2µg/ml respectively. KGVA 3 extract showed lower antioxidant activity, with an IC50 value of 38.5µg/ml. These findings suggested that KGVA 1 and KGVA 2 extracts have potent antioxidant activity, which may help protect cells against oxidative stress. The reducing power assay measures the ability of the

extracts to donate electrons and reduce oxidative stress. The results showed that KGVA 1 and KGVA 2 extracts exhibited similar reducing power to ascorbic acid, with IC50 values of 52.9 and 53.8µg/ml, respectively. KGVA 3 extract showed lower reducing power, with an IC50 value of 79.2µg/ml. These findings suggested that KGVA 1 and KGVA 2 extracts have significant reducing power, which may contribute to their antioxidant activity.

The high DPPH scavenging activity observed in the extracts of *C. americanum* L. may be attributed to the presence of isoquinoline alkaloids, which are known to possess significant antioxidant potential. The antioxidant activity of these compounds is thought to be driven primarily by their proton affinity (PA), which plays a crucial role in the mechanism of sequential proton loss electron transfer (SPLET) (Dung *et al.*, 2020). The present study of DPPH assay measures the ability of the extracts to scavenge free radicals. The results showed that KGVA 1 extract exhibited the highest antioxidant activity, with an IC50 value of 28.8µg/ml, which is lower than that of Vitamin C (34.3µg/ml). KGVA 2 extract showed similar antioxidant activity to Vitamin C, with an IC50 value of 34.3µg/ml. KGVA 3 extract showed slightly lower antioxidant activity, with an IC50 value of 35.2µg/ml. These findings suggested that KGVA 1 extract has potent antioxidant activity which may help protect cells against oxidative stress. The antioxidant activities of the KGVA extracts were compared across the three assays. The results show that KGVA 1 extract exhibited the highest antioxidant activity in all three assays, followed by KGVA 2 extract. KGVA 3 extract showed lower antioxidant activity in all three assays. These findings suggest that KGVA 1 extract has the most potent antioxidant activity among the three extracts. The antioxidant activity of the KGVA extracts was likely due to the presence of bioactive compounds that can scavenge free radicals, donate electrons, and reduce oxidative stress. The extracts may contain phenolic acids, flavonoids and other polyphenolic compounds that have been shown to have antioxidant activity. Further studies are needed to identify the specific bioactive compounds responsible for the antioxidant activity of the KGVA extracts.

CONCLUSION

The present study provides a comprehensive analysis of *Crinum asiaticum* bulb extracts, revealing their potential as a source of bioactive compounds with significant antioxidant activities. The novelty of this study lies in the detailed phytochemical analysis and evaluation of biological activities of *C. asiaticum* bulb extracts, which has not been previously reported.

Specifically, the study demonstrates the importance of optimizing extraction conditions to maximize the yield of bioactive compounds including polyphenolic compounds, alkaloids, glycosides and phenolic acids. This study provides new insights into the potential of *C. asiaticum* bulb as a source of natural products with medicinal properties and the findings have significant implications for the development of natural products for the prevention and treatment of various diseases. Overall, this study contributes to the growing body of research on the phytochemical composition and biological activities of *C. asiaticum* bulb extracts and suggests that *C. asiaticum* bulb extracts may be a potential source of natural remedies for various health conditions.

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