



## Preliminary phytochemical screening, quantification of total phenols and flavonoids and antioxidant potentiality of *Capparis brevispina* dc leaf extract

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### Abstract

*Capparis brevispina* DC is an important medicinal plant of India, which belongs to family Capparaceae. The plant is commonly known as Indian caper, it is a shrub with thorny stipules, coriaceous leaves and white colour flowers while, shape of berries is ovoid to ellipsoid. The plant was found to have medicinal properties and hence used as a traditional medicine in India.

**Method:** The leaves were dried in oven and extracted using maceration cold extraction method in methanol and di ethyl ether solvent. Phytochemical analysis of both the plant extract were done. The plant extract was then evaluated for its antioxidant activity using DPPH assay.

**Result:** The result showed presence of alkaloid, flavonoid, glycosides, protein, terpenoids, cardiac glycoside, steroids, phenols and tannins. The total phenolic content ( $266.66 \pm 6.009$ ), total flavonoid content ( $138.33 \pm 1.666$ ) and antioxidant activity (DPPH assay, IC<sub>50</sub>  $133.422 \pm 0.464$  for standard and  $252.832 \pm 1.727$  for sample (plant extract) was evaluated in methanolic leaf extract of plant. The result showed that plant exhibits antioxidant activity.

**Conclusion:** The DPPH assay showed that the plant has antioxidant activity which can be further evaluated for treating various diseases (e.g., cancer treatment).

The present study was carried out to evaluate the phytochemical and antioxidant activity of leaves of *CB* using methanol and diethyl ether as solvent and diethyl ether for only preliminary screening.

**Keywords:** *Capparis brevispina* dc, phytochemical analysis, total phenolic content, total flavonoid content, antioxidant activity, DPPH

### Introduction

Since ancient time plant plays a significant role in treatment of human diseases and disorders. The property of curing diseases is due to the presence of various bioactive components in the plant. These bioactive constituents are the source of medicine from ancient times <sup>[1]</sup>. Bioactive components are organic compounds with therapeutic properties that are obtained by plants through primary and secondary metabolic processes <sup>[2]</sup>. They are not yet perfectly classified only due to their various forms and structures, but they are also classically studied as two types primary and secondary based on their function in plant metabolism. Common sugars, amino acid, proteins, purines, pyrimidines of nucleic acids and chlorophylls are treated as primary metabolites while alkaloids, flavonoids, steroids, saponins, phenols, glycosides and terpenoids are known as secondary metabolites <sup>[3]</sup>.

Primary metabolites are directly involved in growth and development of plants and are widely distributed in nature in one or the other forms. They play a vital role in plant metabolic process such as respiration, photosynthesis, nutrient assimilation and are also used as food additives and raw material for industries. On the other hand, secondary metabolites work as biocatalysts and are not directly

involved in the plant metabolic process <sup>[4]</sup>. Secondary metabolites are known to be not important for normal growth and development of plant. There are many health benefits related with phytochemicals <sup>[5]</sup>. Alkaloids, flavonoids, tannins, saponin and cardiac glycosides are most important secondary metabolites and are synthesized in secondary metabolic process. They are the key source of pharmaceutical industries due to their medicinal properties <sup>[4]</sup>. Various pathways are involved in synthesis of secondary metabolite including tricarboxylic acid cycle (TCA), methylerythritol phosphate (MEP) pathway, shikimic acid or aromatic amino acid and mevalonic acid pathways <sup>[6]</sup>. According to Saxena *et al*, (2013) there are many phytochemicals present in plant such as alkaloids, flavonoids, steroids, phenols, glycosides and saponins out of all these the largest and most ubiquitous plant metabolite group is phenols <sup>[7]</sup>.

Glycosides, saponins, flavonoids, and alkaloids etc are antibiotics or antimicrobial substances present in plant but due to lack of knowledge and techniques they are not well evaluated. Every secondary metabolite has their own functions such as flavonoid have anticancer property, tannin having antimicrobial activity, antifungal activity in saponin and alkaloid against HIV infection <sup>[4]</sup>.

Capparaceae is a family which consist of herbs or shrubs, rarely small trees. Capparis is an important genus of Capparaceae family. Capparis species are also known as caper plants and are important for their valuable nutrients and physiological function of bioactive compounds [8]. The classification of genus Capparis is on the basis of its morphological characters such as shape of leaf, flowers and spines [9]. Genus Capparis contains nearly 270 species and 40 of its species are found in India. The species from this genus were reported to treat various disease such as tuberculosis, diabetes, hypercholesterolemia, snakebite, etc. Approximately around 400 phytochemicals are reported from genus Capparis. The genus consists species such as *Capparis spinosa*, *Capparis zeylanica*, *Capparis decidua*, *Capparis ovate* and many more including *Capparis brevispina*. The plant is commonly known as Indian caper, it is a shrub with thorny stipules and coriaceous leaves and white colour flowers while shape of berries is ovoid to ellipsoid [10]. The plant is effective against hepatoprotective activities and widely used as tonic, stomachic and also for healing wounds [11]. The main pollinators are birds and butterflies [12].

The present study focused on the phytochemical analysis and antioxidant effect of leaves of *Capparis brevispina*.

## Materials and Methods

### Collection and Identification

The leaves of the plant *Capparis brevispina* were collected from Gujarat college campus, Ahmedabad, Gujarat. The leaves were collected in the month of January 2021 (latitude 23°01'22.8"N and longitude 72°33'57.6"E). Then plant identification and pre-treatment were carried out.

### Plant Material

The freshly collected leaves were washed with tap water and rinsed with distilled water. The leaves were now oven dried at 70 degree Celsius for 5 hours, after drying it was ground into a uniform fine powder. The powder was stored in airtight bottle for further investigation.



**Fig 1:** *Capparis brevispina* DC a. shrub b. leaf c. flowers

## Preparation of Extract

The dried powder material was extracted successively with methanol and diethyl ether using maceration cold extraction method. 5gm powder was dissolved in 50ml of solvent (methanol and diethyl ether) separately in orbital shakers for 24 hours at 112rpm at normal temperature. Then extract was filtered through whatmann filter paper no.1 in a pre-weighed petri-plates. After filtration, the extra solvent was evaporated and petri-plates stored at 4° C for further analysis. Then the yield value of crude extract was calculated using given standard formula.

$$\text{Yield (\%)} = \frac{\text{Weight of dry extract}}{\text{Weight of plant powder}} \times 100$$

## Preliminary Phytochemical Screening

Phytochemical analysis was performed to identify potent phytoconstituent of *Capparis brevispina* leaves extract. The crude methanolic extract of *C. brevispina* leaves were tested for the presence of alkaloids, flavonoids, tannins, saponins and glycosides. The phytochemical screening was done through following standard methods by shaikh and Patil [13].

### Test for Alkaloids

- 1. Mayer's Test:** 2ml plant extract was treated with 1ml Mayer's reagent. The presence of white creamy precipitate indicates presence of alkaloids.
- 2. Wagner's Test:** 2ml Wagner's reagent when added to 2ml plant extract gives red brown precipitate, this red brown precipitate indicates presence of alkaloid.
- 3. Hager's Test:** 1-2ml Hager's reagent when added to 2ml plant extract, an appearance of yellow precipitate indicates presence of alkaloid.
- 4. Dragendroff's Test:** 2ml Dragendroff reagent mixed with 1ml plant extract, presence of orange precipitate indicate that alkaloid is present.

### Test for Flavonoid

- 1. Alkaline Reagent Test:** few ml plant extract treated with 3ml of 2% NaOH shows yellow colour, then addition of dil. H<sub>2</sub>SO<sub>4</sub> shows yellow colour disappearing this indicates the presence of flavonoid.
- 2. Lead Acetate Test:** few drops of 10% lead acetate was added to few ml plant extract, an appearance of yellow precipitate indicates presence of flavonoid.

### Test for Glycosides

- 1. Libermann's Test:** 2ml chloroform and 2ml acetic acid was added to 2ml plant extract, then conc. H<sub>2</sub>SO<sub>4</sub> cooled on ice shows colour change from violet to blue to green, this colour change indicates presence of glycoside.
- 2. Borntrager's Test:** 3ml chloroform mixed with 2ml extract and shaken well, then add 10% ammonium solution gives pink colour, appearance of pink colour shows presence of glycosides.

- Keller-Kilani Test:** Plant extract when treated with 1ml glacial acetic acid and 2 drops of 2% FeCl<sub>3</sub> solution then the whole mixture pour into another test tube containing 1ml conc. H<sub>2</sub>SO<sub>4</sub>, formation of 2 layers is seen upper red brown and lower blue green this indicates presence of glycoside.

#### Test for Proteins

- Millon's Test:** 2ml plant extract when treated with 2ml Millon's reagent, an appearance of white precipitate indicates presence of protein.
- Biuret Test:** 0.5ml 2% CuSO<sub>4</sub> added to 2ml extract and then 1ml 95% ethanol was added at last 1 KOH pellet, formation of pink colour of each layer indicates presence of protein.

#### Tests for Phenols and Tannins

- Ferric Chloride Test:** 2ml plant extract when treated with 1-2 drops of 5% FeCl<sub>3</sub> appearance of dark green colour indicates presence of phenols and tannin.
- Lead Acetate Test:** 2ml extract when treated with 0.5 ml of lead acetate gives white precipitate, presence of white precipitate indicates presence of phenol and tannin.
- Folin-Ciocalteu:** 0.5-1ml plant extract when treated with 1ml folin-Ciocalteu gives blue-green colour, appearance of blue-green colour indicates presence of phenol and tannin.

#### Test for Terpenoids

- Copper Acetate Test:** 1-2 drops of copper acetate solution when added to few ml plant extract gives emerald green precipitate, emerald green precipitate indicates the presence of terpenoid.
- Salkowski's Test:** Plant extract treated with 2ml chloroform and 3ml conc. H<sub>2</sub>SO<sub>4</sub> gives formation of red brown colour ring, red brown colour ring indicates presence of terpenoid.

#### Test for Cardiac Glycosides

- Cardenolide Test:** 1ml pyridine was added to 2ml extract with 1ml 20% sodium nitroprusside, appearance of pink or red colour indicates the presence of cardiac glycosides.

#### Test for Steroids

- Salkowski's Test:** 2ml extract with few ml chloroform shaken well and then added conc. H<sub>2</sub>SO<sub>4</sub> gives red colour, red colour shows presence of steroid.
- Libermann's Burchard's Test:** 2-3ml of acetic anhydride solution was added to 1ml extract, then 2ml H<sub>2</sub>SO<sub>4</sub> was added to mixture an appearance of green or violet colour indicates the presence of steroid.

#### Determination of Total Phenolic Content

**Chemicals:** Folin-Ciocalteu reagent, Gallic acid (standard), 20% Sodium carbonate, Distilled water, Methanol, Plant extract

#### Procedure for Total Phenolic Content

The total phenolic content of methanol leaf extract of *Capparis brevispina* DC was determined by using folin-Ciocalteu reagent method with some modification. The method used was similar as performed by Li *et al.*, (2015) [14] with some modification. Gallic acid was used as standard. The total phenol content in extract was expressed in mg/g Gallic Acid Equivalents (GAE). The gallic acid and plant extract was separately prepared in methanol at a concentration of 1mg/5ml. The reaction mixture was prepared by mixing 1ml folin-Ciocalteu to 1 ml plant extract and dissolve in 10ml distilled water. Then 4ml of 20% sodium carbonate add to make the total volume 25ml with distilled water in each test tube. Then test tube was allowed to incubation for 30 minutes at normal room temperature. The entire test was performed in triplicates. The absorbance of sample was measured at 765nm and mean value of absorbance was obtained. The same procedure was repeated for the solution of Gallic acid (Standard). The results were expressed as mg gallic acid equivalent (GAE) per gram of extract. Thus, total phenolic content can be determined by blue colour producing by folin-ciocalteu reagent in presence of polyphenols which measured spectrophotometrically.

#### Determination of Total Flavonoid Content

**Chemicals:** Quercetin (standard), 10% Aluminium chloride, 1M Potassium acetate, Distilled water, Methanol, Plant extract.

#### Procedure for Total Flavonoid Content

The total flavonoid content of methanol leaf extract of *Capparis brevispina* DC was analysing aluminium chloride colorimetric method used by CC Chang *et al.*, (2002) [15] with some modifications. In this method quercetin was used as standard (1mg/5ml). The total flavonoid content in extract was expressed in mg/g Quercetin equivalent. The Quercetin and plant extract were separately prepared in methanol at a concentration of 1mg/5ml and 100µL 10% AlCl<sub>3</sub> (aluminium chloride), 100µL 1M CH<sub>3</sub>COOK (potassium acetate) was mixed with plant extract which then dissolved in 4.8ml of distilled water. Then the test tubes were incubated for 30minutes at room temperature. All the test were performed in triplicates. The absorbance of standard and sample were measured at 415nm. The results were expressed as mg Quercetin equivalent (QE) per gram of plant extract.

#### Antioxidant Assay

**Chemical:** Ascorbic acid (standard), DPPH (2, 2-diphenyl-1-picrylhydrazyl) Methanol, Plant extract

#### Free Radical Scavenging Activity-DPPH Assay

Free radical scavenging activity of *Capparis brevispina* DC leaf extract was determined by DPPH assay. DPPH (2, 2-diphenyl-1-picryl-hydrezylyl) was used as free radical to determine antioxidant activity. The method used was similar to previously done by M Kosanic *et al.*, (2014) [16] with some modification. For the preparation of DPPH, the 4 mg (0.004%) of DPPH dissolving in 100mL methanol. 2ml of prepared DPPH (2,2-diphenyl-1-picrylhydrazyl) mixed with plant extract and incubated for 20-30 minutes in dark at room temperature. All the test were performed in triplicates. The absorbance of sample was measured at 517nm.

Ascorbic acid was used as standard solution. The results were expressed by IC<sub>50</sub> value. Radical scavenging activity was compared with inhibition concentration at 50% inhibition (IC<sub>50</sub>).

Following formula was used for calculating DPPH Scavenging activity:

$$\text{DPPH scavenging activity (\%)} = \frac{[\text{absorbance of blank} - \text{absorbance of sample}]}{\text{absorbance of blank}} \times 100$$

### Statistical Analysis

The results were expressed as means  $\pm$  standard deviation (SD) of three triplicates. The data was analysed using Microsoft excel tools.

### Results and Discussion

The yield value of both the extract was calculated by using the formula used by author Abdullah *et al.*, (2012) [17]. Yield percentage of both the extract is shown below in table 1. The yield percentage of leaf extract in methanol was 11.1% and in di-ethyl ether was 1.27%. Nature of crude extract is shown in table 2.

**Table 1:** Percentage Yield of *Capparis brevispina* Leaf Extracts

Sample	Yield %
Methanol	11.1%
Diethyl ether	1.27%

Percentage yield of *Capparis brevispina* leaf extract in two different solvent methanol and diethyl ether

**Table 2:** Nature of Crude Leaf Extract of *Capparis brevispina*

Sample	Colour	Consistency
Methanol	Light green	Sticky
Di-ethyl ether	Light green	Sticky

Nature of crude leaf extract of plant *Capparis brevispina* in two different solvent methanol and diethyl ether.

### Preliminary Phytochemical Screening Test

The qualitative phytochemical screening was carried out on methanol and diethyl ether leaf extract of *Capparis brevispina*. The result showed presence of alkaloids, flavonoid, glycosides, proteins, phenols, tannin, terpenoids, cardiac glycosides, steroids in both the extract of plant *Capparis brevispina* (methanol and diethyl ether). The result is tabulated in the below given table 3.

**Table 3:** phytochemical analysis leaf extract of *capparis brevispina*

Sr.no.	Phytochemical	Test	Methanol	Diethyl Ether
1.	Alkaloids	Mayer's test	+	-
		Wagner's test	+	-
		Hager's test	+	+
		Dragendroff's test	+	+
2.	Flavonoid	Alkaline reagent test	+	+
		Lead acetate test	+	+
3.	Glycosides	Libermann's test	+	-
		Borntrager's test	-	-
		Keller-kilani test	-	+
4.	Proteins	Millon's test	+	-
		Biuret test	-	-
5.	Phenols/ Tannins	Ferric chloride test	-	+
		Lead acetate test	+	+
		Folin-ciocalteu test	-	-
6.	Terpenoids	Copper acetate test	+	+
		Salkowski's test	+	-
7.	Cardiac glycosides	Cardenolide test	+	+
8.	Steroids	Salkowski's test	-	-
		Libermann's- burchard's test	+	+

+ Symbol indicates the presence and - indicates the absence of phytochemical in two different solvent leaf extract of plant *Capparis brevispina*.

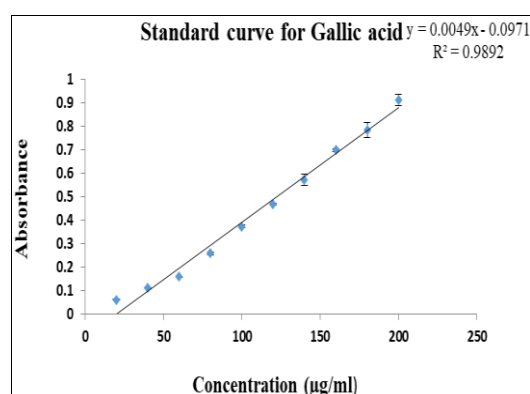
### Quantitative Determinations

The quantitative assay was performed for phytochemicals (phenols and flavonoid) and antioxidant activity in methanol extract using UV-Vis spectrophotometer with standard protocol.

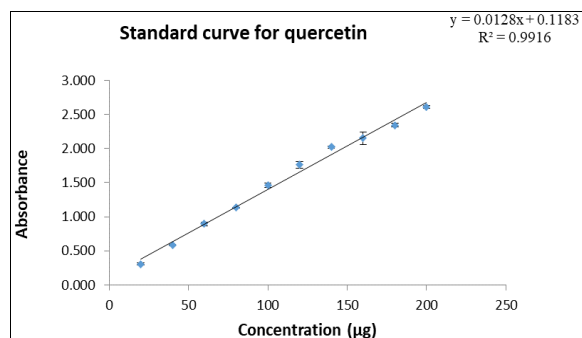
### Estimation of Total Phenolic Content and Total Flavonoid Content

Plant phenols and flavonoids are the major group of compounds that act as primary antioxidant or free radical scavenging agent. Therefore it is important to determine total phenol content in plant extracts. For estimation of antioxidant capacity, it is important to estimate total phenolic content and total flavonoid content present in extract. The most abundant phenol and flavonoid which possess significant antioxidant properties are Gallic acid and Quercetin respectively thus used as standard in the present work. The methanolic extract of *C. brevispina* leaf extract showed the total phenolic and total flavonoid content were

266.66 $\pm$ 6.009 and 138.33 $\pm$ 1.666 respectively. Figure 2 and 3 shows the graph of standard solution for phenolic and flavonoid content.



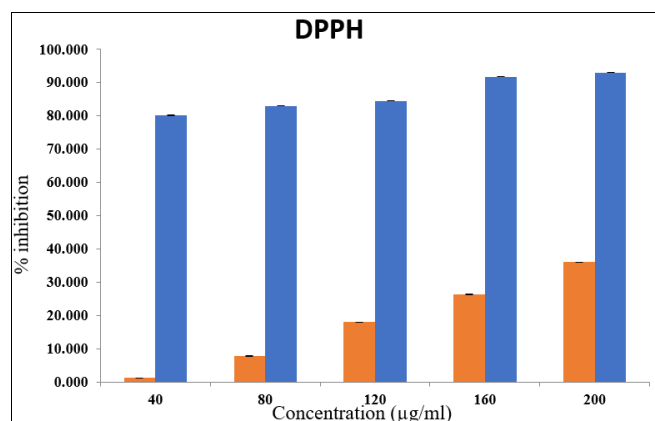
**Fig 2:** Standard curve of different concentration of gallic Acid (µg/ml) and their respective absorbance



**Fig 3:** Standard curve of different concentration of quercetin ( $\mu\text{g/ml}$ ) and their respective absorbance

### Estimation of Antioxidant Activity

The free radical scavenging activity of the methanolic extract of *Capparis brevispina* has been tested by DPPH radical method using DPPH (2, 2-diphenyl-1-picrylhydrazyl) as standard. DPPH is act as free radical and it changes colour from purple to yellow when react with radical scavenger. The result was analysed as IC<sub>50</sub> value calculated from the graph obtained by plotting the %inhibition against concentration value and expressed in mg/ml of the extract. The result showed the significant antioxidant potentiality of sample ( $252.832 \pm 1.727$ ) when compared with standard ( $133.422 \pm 0.464$ ). Figure 4 shows percentage inhibition of standard and plant extract.



Graph shows comparison between % inhibition of standard and sample solution

- In dication of % inhibition for standard as ascorbic acid,
- In dication of % inhibition for sample (leaf extract of plant)

**Fig 4:** % Inhibition activity of ascorbic acid (standard) and *Capparis brevispina* extract

Phytochemical analysis conducted on plant extract of *Capparis brevispina* revealed presence of many bioactive components which are known for exhibiting medicinal activities. Analysis of plant extract revealed the presence of alkaloid, flavonoid, glycoside, phenols, tannins, proteins, cardiac glycosides, terpenoids and steroids. Methanolic leaf extract of *Capparis brevispina* showed better results as compared to diethyl ether and other solvents like hexane, chloroform, ethanol and water extract of plant leaves [11]. In *Capparis zeylanica* Linn, protein and steroids were found to be absent in methanolic extract [18], while were present in *Capparis brevispina*. Similarly, *Capparis spinosa* L shows the significant result in ethanol and water extract, among all the extract including hexane, chloroform and ethyl acetate [19]. The result suggested that alkaloid was absent in hexane, chloroform and ethyl acetate while present in ethanol and

water extract [19]. Similarly, *Capparis seiparia* L leaf extract lacks the alkaloid, flavonoid, steroid, terpenoids and phenolic compound in ethanol and water extract [20].

The total phenolic content ( $266.66 \pm 6.009$ ) was found to more than flavonoid content ( $138.33 \pm 1.666$ ) in the methanolic plant extract of *Capparis brevispina*. In other solvent like water, hexane, ethanol and chloroform the total phenolic content was found to more than flavonoid content, although water has the highest total phenolic and flavonoid content in comparison to other solvent and lowest was for chloroform [11]. Like-wise in *Capparis spinosa* L among the methanolic and aqueous leaves extract, methanolic extract showed higher amount of phenolic and flavonoid content than in aqueous extract [21]. In *Capparis decidua* methanolic leaf extract the total phenolic content was found  $286.51 \pm 4.62$  while in *Capparis brevispina* it was  $266.66 \pm 6.009$  which is low as compared to that of *Capparis decidua* (forsk.) edgew [22]. The total flavonoid content of methanolic extract of *Capparis mucronifolia* ( $76.1 \pm 1.74$ ) was high in comparison to that of *Capparis cartilaginea* ( $33.77 \pm 0.76$ ) [23] but both having low amount in comparison to *Capparis brevispina*.

The antioxidant activity of methanolic leaf extract of *C. brevispina* evaluated using DPPH method. In this method, DPPH become a stable diamagnetic molecule or a stable free radical after accepting an electron or hydrogen radical. Th reason for antioxidant activity is the ability of donating hydrogen by DPPH [24]. In the present study, the IC<sub>50</sub> value of sample and standard were compared and result showed that, the plant extract exhibit better antioxidant activity. IC<sub>50</sub> value of methanolic plant extract was  $252.832 \pm 1.727$ , while in other solvent it was ethanol (52.37%), chloroform (51.14%), water (50.79%) and in hexane (48.82%). The result suggested that among the all solvent hexane showed lowest scavenging activity while ethanol shows highest scavenging activity [11]. This variation may be due to different phenolic content in compound [11]. In twigs of *Capparis decidua* L different solvent were used for antioxidant activity in which chloroform and ethyl acetate extract showed lowest DPPH scavenging activity while petroleum ether and butanol showing highest DPPH scavenging activity [25]. According to Kumar *et al*, (2019) *Capparis zeylanica* possesses effective antioxidant potentiality against different solvent concentration ranging from 10-160 $\mu\text{g/ml}$  [26]. The methanolic extract of *Capparis zeylanica* possess highest activity then aqueous extract, acetone, ethyl acetate. It also shows better result than *Capparis brevispina*.

### Conclusion

The phytochemical screening showed that *Capparis brevispina* leaf extract contain a wide range of phytochemicals including alkaloid, flavonoid, glycosides, proteins, steroids, cardiac glycoside, terpenoids, phenols and tannins. Among the all bioactive, phenol and flavonoid are important phytoconstituents for scavenging potentiality of the plant. The phenolic content was more as compared to flavonoid content in this plant extract. The methanolic extract of *C. brevispina* showed the significant antioxidant potentiality due to presence of phenols and flavonoids. Thus, the present work suggests that *C. brevispina* possesses potential pharmaceuticals and tremendous medicinal properties, but unfortunately it has not drawn the deserving attention and shall be explored further to investigate for

their various pharmacological aspects. The available data Expressed that the *C. brevispina* needs to be attended attentively for its hidden potential.

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