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Research Article

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Bioactive compounds, Antioxidant and Antimicrobial Attributes of *Cordia Sinensis Lam*

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Abstract

The aim of this study is to discover the efficacy of various parts of *Cordia sinensis* plant against some particular pathogens that are recognized to cause diseases and to check antioxidant and bioactive compounds from different parts of plant *C. sinensis*. Plant extracts were examined by quantification of phytochemical compounds and antimicrobial activity. Phytochemicals and several secondary metabolites were quantified and also qualitatively analyzed in *C. sinensis* extracts such as phenolic compounds, flavonoids, alkaloids, tannins, steroids, glycosides, and saponins. Similarly, biochemical primary metabolites like protein, total sugar, and reducing sugar were also estimated in different parts of *C. sinensis* and these results correlated with antimicrobial activity. This study reveals that acetone extracts of stems, leaves, and roots of the *C. sinensis* showed excellent antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* and also against fungal species *Aspergillus niger*, *Candida albicans*, and *Penicillium notatum*.

Keywords: Antimicrobial activity, Antioxidants, Bioactive Compounds, *Cordia sinensis*, Medicinal Plant

Introduction

Kingdom Plantae is considered the most important source of all pharmaceutical's world. This kingdom is of utmost use for the cure of numerous diseases (Ajayi *et al*., 2011). All over the world medicinal plants are used for the treatment of various types of human and animal diseases (Kumar *et al.*, 2021). Peoples who live in the countryside have the first choice to use herbal medicines for health care. It has been noticed that in those countryside regions where conventional medicines are frequently available, the interest of people for taking natural medications derived from plants has been increased. Medicinal plants comprise numerous bioactive components, including phytochemicals, which have various physiological effects on humans (Khan *et al*., 2021 and Marini *et al.*, 2018), which prevent several types of diseases including diabetes, heart disease and cancer (Kumar *et al.*, 2021). Plant-based natural nutritional supplements, herbal teas, pharmaceuticals, cosmetics and several other health-stimulate products significantly increased in recent years (Naikoo *et al.*, 2019 and Sharma *et al*., 2019).

The genus *Cordia* comprises about 300 species belonging to the family Boraginaceae and most of the species are trees or shrubs (Oza & Kulkarni, 2017). *Cordia sinensis* Lam (C *sinensis* Lam) is a multi- stemmed tree, commonly found in arid to semiarid regions of different countries including Pakistan, India and Sri Lanka (Gumgumjee and Hajar 2015 and Verdcourt 1991). Cordia species, besides ethnobotanical and ethno-medicinal features, are prominent for their various range of bioactivities and secondary metabolites (Rahman and Akhtar, 2016). Researchers have believed that free radicals are produced in the body due to changes in community living style, poor eating habits, and age factors. Antioxidants are compounds that defend the human immune system from free radicals that produce various chronic syndromes like asthma, anemia, inflammation and ageing (Prabu *et al*., 2019). Phytochemicals like flavonoids, terpenoids and phenols are considered to be anti-free radicals (antioxidants) (Mega and Swastini, 2010). These compounds are considered as antioxidant sources (Surjanto *at el*.,2014) and safe for consumption (Batubara *et al*.,2016). The plants are considered antibiotic sources due to the presence of bioactive compounds like alkaloids, phenols, glycosides, flavonoids, and Saponins. These bioactive constituents showed anti-inflammatory, antifungal

activity, and molluscicide activity (Ajayi *et al.*, 2011). In the modern world challenges for scientists are increasing in the medical side for discoveries of new sources and scientific methods for diagnosing and treating the diseases. Because most of the microbes showed resistance, it is a need for time to explore alternative sources of antibiotics. To resolve this type of problem, plants are a substitute source due to the presence of a wider variety of phytochemicals that can be used for the treatment of various diseases. The species *C. sinensis* has unique medicinal importance and very little literature available on this plant species as a medicinal plant and need more investigation on different phytochemicals. The purpose of this study is to investigate different phytochemicals such as antioxidants, terpenoids, flavonoids, steroids, glycosides, saponin, tannins, phenolics, sugars and proteins from different parts including Leaves, Stems and Roots qualitatively and quantitatively from *C. sinensis* which can be used to cure different diseases.

Materials and Methods

Collection and preparation of plant sample: All chemicals used in this study were bought from Sigma and Merck. All chemicals used were of analytical grade.

The different parts of the plant (stems, roots and leaves) were collected from the healthy *Cordia sinensis* Lam plant at Allama I.I Kazi Campus, Sindh University, Jamshoro, Pakistan. The plant species was authenticated at the herbarium unit of the Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan. The roots, stems and leaves were brought to the Laboratory carefully in plastic bags, washed with distilled water and dried at room temperature.

The collected samples of roots, stems, and leaves of C. *sinensis* were dried at room temperature for one week in the shade and were ground to a fine powder. 20 g of powder sample was immersed in distilled water and 90% aqueous acetone for 24 h at room temperature. The immersed plant samples were ground with glass powder, squeezed through cheesecloth, and centrifuged at 4°C at 7000 rpm (refrigerated centrifuge, Kubota TMAX-CL5R, Japan), same procedure was repeated twice, and the final volume was made up to 100 ml with distilled water and 90% aqueous acetone. The sample was stored at 4°C for further analysis. From prepared 20% aqueous acetone and water extracts, several phytochemicals / biochemicals tests including antioxidants, phenolic compounds, flavonoids, protein, total sugar and reducing sugar were investigated by U.V.-Vis. Spectrophotometer (UV-752). In addition, the antimicrobial activity of under studied plant extracts was tested versus various pathogenic bacterial and fungal species.

Qualitative screening and quantification of plant bioactive compounds: The aqueous acetone and water extracts of leaves, roots and stems of *C. sinensis* were analyzed qualitatively for confirmation of different metabolites such as tannin, flavonoids, alkaloids, terpenoids and saponin by their respective reported methods (Rahu *et al.*, 2021). The presence of different metabolites in the extract were confirmed through development of different colours during the reactions. Total antioxidant activity was performed quantitatively from acetone and water extracts of *C. sinensis* using different concentrations of α-Tocopherol used as a standard by the reported method (Arabshahi *et al*., 2007). The total phenolics and flavonoids from acetone and water extract of leaves, roots and stems of *C. sinensis* Lam were determined quantitatively by reported methods (Djeridane *et al.*, 2006 and Yasoubi *et al*., 2007).

Quantification of total protein, total sugar and reducing sugar: The concentration of total protein was detected from 20% acetone and water extract of leaves, roots and stems of *C. sinensis* plant (Classics Lowry *et al*., 1951) by using Bovine serum albumin as standard. Total sugar content from acetone and water extracts of leaves, roots and stems of *C. sinensis* Lam was determined by the reported method of Montgomery (1961). However, reducing sugar content was recorded from acetone and water extracts of leaves, roots and stems of *C. sinensis* Lam by reported method of Miller (1959).

Bacterial and Fungal Culture: The different bacterial species (*Staphylococcus aureus* IBGE-012, *Pseudomonas aeruginosa* IBGE-031 *and Klebsiella pneumonia* IBGE-014) and fungal species (*Aspergillius niger* EFRL- FC-024, *Candida albicans* EFRL-FC- 011 and *Penicillium notatum* EFRL-FC-030) were obtained from Microbial Bank of Institute of Biotechnology & Genetic Engineering, University of Sindh, Pakistan for antimicrobial activity. These strains were cultured for luxurious growth on nutrient agar medium for 24 h for bacterial species and 7 days for fungal species. The strains dilutions were prepared in sterilized distilled water with a full loop of inoculums. The inoculated both bacterial and fungal cultures were incubated at 37°C. The standard antibiotics were used as positive control (Piperacillin / Tazobactam 110 µg, Avelox 5 µg, Oxacillin 1 µg, Erythromycin 15 µg, and Ofloxacin 5 µg). Antibacterial and antifungal activity was tested against selected under-study bacterial and fungal species by well diffusion method as reported by (Imran *et al*.,2011). 20 µl volume of aqueous acetone and water extracts were applied in each well and also, pure acetone (without aqueous dilution) in same quantity was applied in well as a control to check the antimicrobial activity.

Statistical analysis: Experimental data are revealed by using three replicates and Mean ± standard deviation (SD). Using SPSS, comparison of variables with water and acetone extract of different parts of *C. sinensis* plant was done. Students T-Test was used for comparison between two means of each variable taking P-value ≤ 0.05 as significant.

Results

Qualitative Analysis of Plant Bioactive Compounds: In the initial step of present study, it was necessary to confirm the presence of different qualitative bioactive compounds in *C. sinensis*' segments. These plant bioactive compounds not only qualitatively screened out but also these bioactive compounds/biomolecules were also quantified and correlated to each other and also with antimicrobial activity against some selected different lethal microbes and found fruitful results.

The efficacious results of tannin were achieved from various parts (leaves, stems and roots) of *C. sinensis* extracts by using the color reaction method qualitatively as depicted in Table.1. It was observed from the development of a greenish color during the reaction qualitatively that each part of *C. sinensis* contained a specific concentration of tannins. The appearance of color intensity proved the presence of tannins in moderate amount (++), both in acetone and water extract of the leaves while low amount (+) in acetone and water extract of stems and roots.

The reagents were amalgamated in the acetone and water extracts of different parts of *C. sinensis*, the associate reddish-brown layer was observed which showed the presence of terpenoids. The results presented in Table 1 illustrates that acetone-extracted samples of leaves contained a moderate amount (++) while stems and roots contained a low amount (+) of terpenoids. On the other hand, water extracted sample of leaves and roots exhibited, a moderate amount (++) but the stems contained terpenoids in low amount (+) during the qualitative detection using the color reaction method.

It was revealed from the results as depicted in Table 1, that water-extracted samples of stems and roots contained a high quantity (+++) of flavonoid as compared to acetone-extracted samples of stems and roots which contained a low quantity (+) of flavonoid. Furthermore, both acetone and water extract of leaves of C. *sinensis* had a low amount (+) of flavonoid.

The reddish-brown colored precipitates were obtained after adding reagents in acetone and water extract of different parts (leaves, stems, and roots) of *C. sinensis* which indicated the presence of alkaloids. The highest color of intensity (+++) was noted in the water extract of all parts of *C. sinensis* but the medium color of intensity (++) was noticed in the acetone extract of the leaves. The lowest color of intensity (+) was produced in the acetone extract of stems and roots as reported in Table 1.

The Saponins test was accomplished from acetone and water extracts of leaves, stems, and roots of *C. sinensis* through the reported color reaction method. After adding the chemicals and a few drops of olive oil to acetone and water extracts, a stable foam was observed that indicated the presence of the Saponins qualitatively. Results of chemical analysis of water extracts of *C. sinensis* leaves revealed the presence of a high amount (+++) of saponins while stems and roots have a moderate amount (++). On the other hand, acetone extract of all parts contained a moderate amount (++) of saponins as exhibited in Table 1.

Table 1. Qualitative Analysis of Bioactive compounds from 20% acetone and water extracts of different parts of *C. sinensis*

Phytochemicals	Extracts of different parts of *C. Sinensis*					
	Acetone			Water		
	Leaves	Stems	Roots	Leaves	Stems	Roots
Tannins	++	+	+	++	+	++
Terpenoids	+	++	+	+	++	+
Flavonoids	++	+	+	++	+++	+++
Alkaloids	+	++	+	+++	+++	+++
Saponins	++	++	++	+++	++	++
Steroids	+++	+++	++	+	++	++
Phenolics	++	+++	++	+	+	+++
Glycosides	++	+++	+++	++	++	++

+++: high amount, ++: moderate amount, +: low amount

Quantitative Analysis of Plant Bioactive Compounds: The antioxidant activity of different parts (leaves, stems and roots) of *C. sinensis* was quantified through the reported method. In the present study, both acetone and water extract showed. maximum quantity in stems (2.19 ± 0.07 mg/mL and 2.12 ± 0.09 mg/mL) whereas least quantity was recorded in roots (1.92 ± 0.1 mg/mL and 1.87 ± 0.124 mg/mL) respectively. On the other hand, both acetone and water extract of leaves possessed significant amount (1.69 ± 0.18 mg/mL and 1.86 ± 0.12 mg/mL) correspondingly as shown in Fig.1.

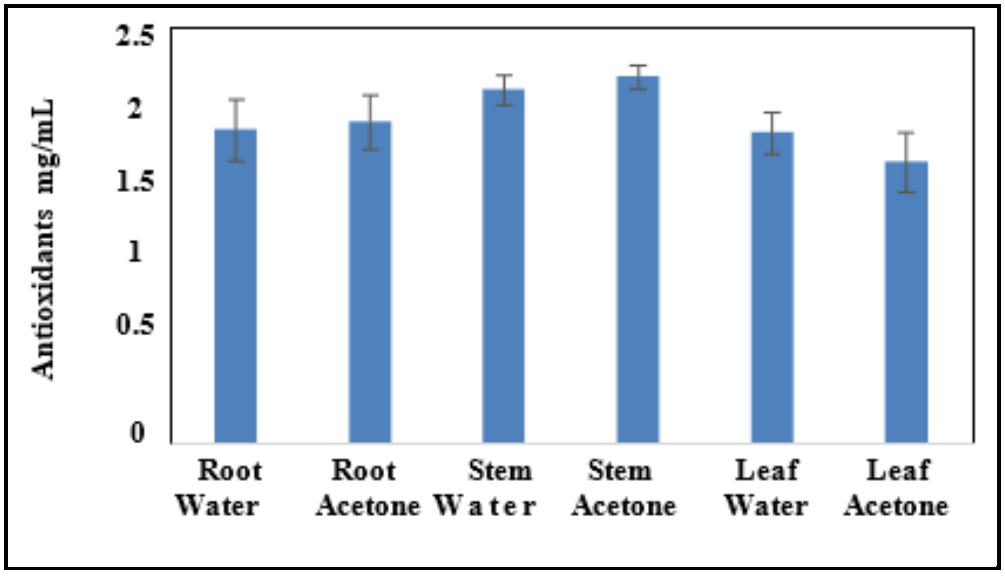

Figure 1. Quantification of Antioxidant activity from extracts of *C. sinensis.*

Total phenolic content was computed from various parts of *C. sinensis* in acetone and water extracts as reported in Fig.2. In the present study, acetone extract showed total phenolic content in roots 7.53 ± 0.19 mg/mL, in stems 6.74 ± 0.18 mg/mL and in leaves 6.07 ± 0.45 mg/mL. whereas water extract contains 7.08 ± 0. 82 mg/mL in roots, 6.59 ± 0. 82 mg/mL in stems, and 5.16 ± 0.52mg/mL total phenolic compounds in leaves. It was observed that phenolic content in acetone root extract was 124% higher than leaves while water root extract showed 137% higher phenolic content than the leaves. The maximum phenolic concentration was noted in the acetone extracted roots sample (7.53 ± 0.19 mg/mL).

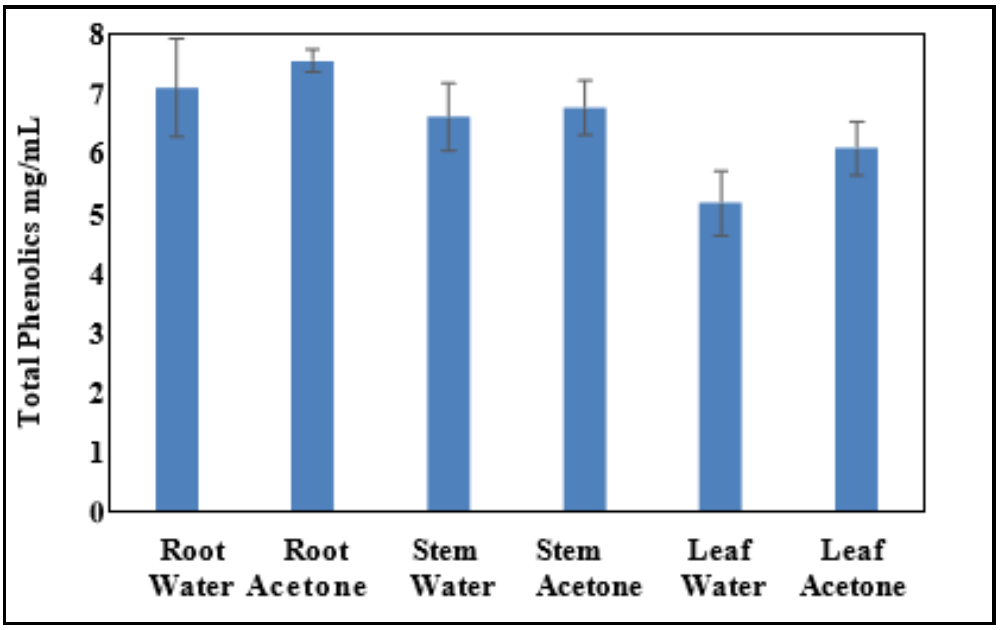

Figure 2. Quantification of Phenolic Compound from extracts of *C. sinensis*

Quantification of the total flavonoid in different segments of *C. sinensis* was studied as exhibited in Fig.3. The highest quantity of flavonoids was estimated in stems extracts of acetone (0.14 ± 0.01 mg/mL) than stems extract of water (0.13 ± 0.01 mg/mL). Similarly, the maximum amount of flavonoids was found in roots extracts of acetone (0.11 ± 0.01 mg/mL) than roots extracts of water (0.08 ± 0.01 mg/mL). On the other hand, the highest amount of flavonoids was noted in leaves extracts of acetone (0.09 ± 0.01 mg/mL) than leaves extracts of water (0.07 ± 0.01 mg/mL). It was revealed from the results that acetone extracts of all parts of *C. sinensis* had high flavonoids contents than water extract.

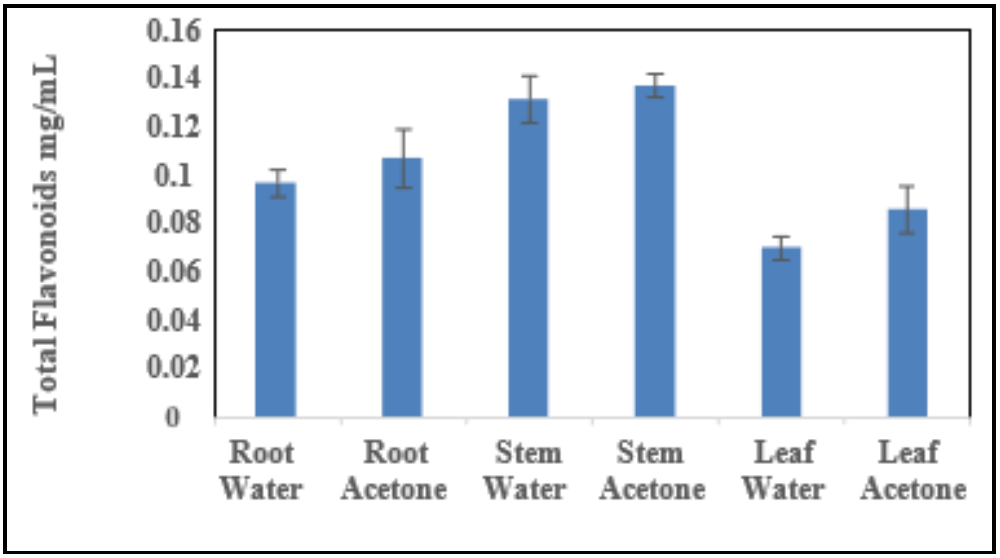

Figure 3. Quantification of Total Flavonoids from extracts of *C. Sinensis*

Quantitative analysis for Total Protein, Total sugar and Reducing sugar: The quantification of total protein was carried out in aqueous and acetone extracts of different parts of *C. sinensis* through the reported method. All segments of *C. sinensis* plant extracts revealed a rich amount of protein (Fig. 4). However, the highest quantity was obtained in the water extract of the stems (2.05 ± 0.12 mg/mL). Similarly, a noteworthy quantity of various parts of *C. sinensis* was also associated to each other, it was noted that moderate range of total protein as in acetone extract of stems, leaves and water extract of leaves 1.78 ± 0.10 mg/mL, 1.72 ± 0.2 mg/mL and 1.76 ± 0.032 mg/mL respectively. Moreover, it was observed that acetone extract of the roots estimated protein 1.56 ± 0.10 mg/mL but slightly low (1.38 ± 0.14 mg/mL) in the water extract of roots.

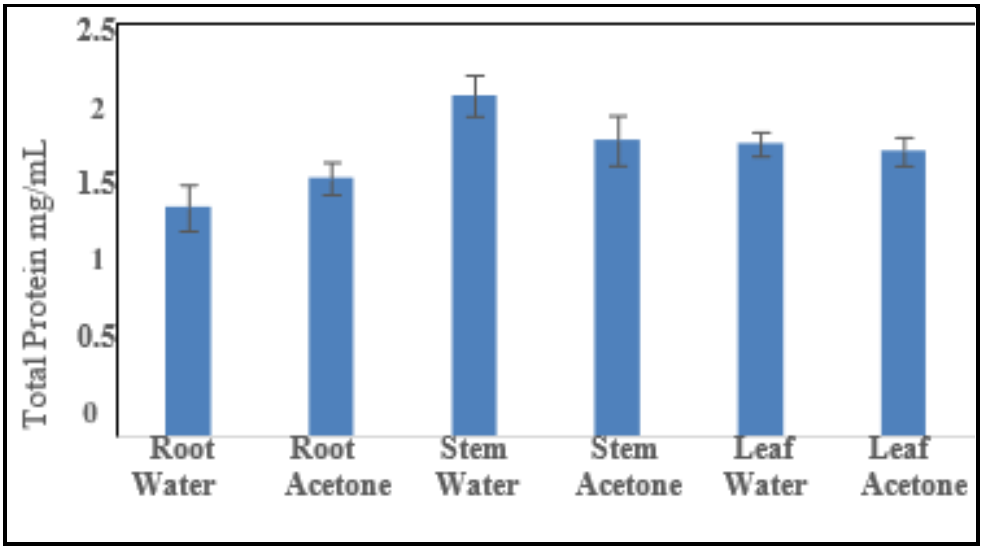

Figure 4. Quantification of Total Protein from extracts of *C. sinensis*

The total sugar was assessed from acetone and water extracts of *C. sinensis*, the results are summarized in Fig. 5. According to the results acetone extract of stems and water extract of stems and roots contained total sugar 167.67 ± 0.17 mg/mL, 169.68 ± 0.52 mg/mL and 167.62 ± 0.12 mg/mL respectively. However, a very impressive but moderate amount of total sugar was also detected in the water extract of leaves (153.68 ± 3.06 mg/mL). Moreover, both acetone extracts of leaves and roots exhibited the lowest amount of total sugar but acetone extract of the leaves had a high amount (119.22 ± 0.33 mg/mL) as compared to the roots (99.22 ± 0.11 mg/mL). The overall results indicated that acetone extracts of different parts *C. sinensis* showed a higher concentration of total sugar as compared to water extract.

The quantification of reducing sugar was done through the reported method. According to the result water extract of roots showed the maximum quantity of reducing sugar (1.96± 0.01 mg/mL) as compared to other part of *C. sinensis*. Nevertheless, a significant amount of reducing sugar was also present in acetone

extract of roots 1.712 ± 0.06 mg/mL, stems 1.66 ± 0.01 mg/mL and water extract of leaves 1.52 ± 0.01 mg/mL. On the contrary the minimum quantity of reducing sugar was found both acetone extract of leaves and water extract of the stems 1.408 ± 0.06 mg/mL and 1.48 ± 0.04 mg/mL respectively as depicted in Fig.5.

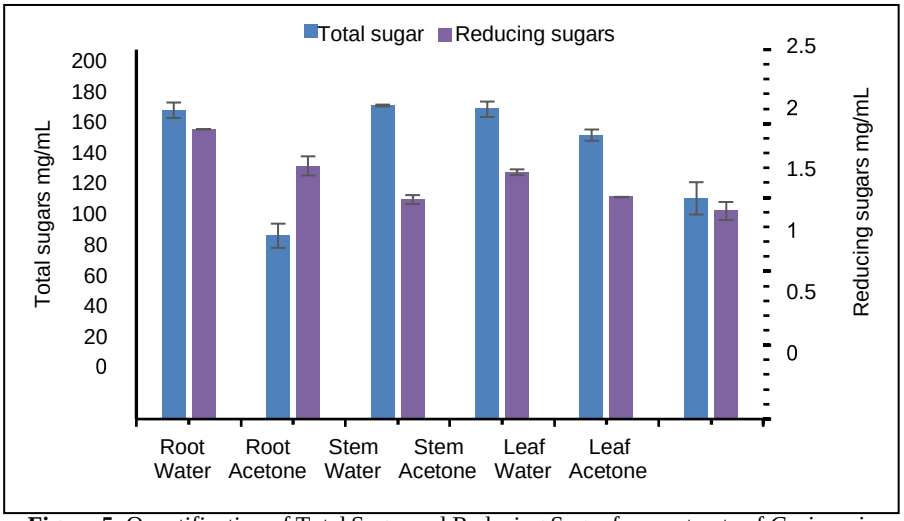

Figure 5. Quantification of Total Sugar and Reducing Sugar from extracts of *C. sinensis*.

The Comparison of variables with water and acetone extract of different parts of *C. sinensis* plant was exhibited in Table-2. The students T-Test used for comparison between two means of each variable taking P-value ≤ 0.05 as significant.

Table 2. Comparison of variables with water and acetone extracts of different parts of *C. sinensis* plant

Variables		water extract	acetone extract
		(Mean± S.D)	**(Mean± S.D)**
Antioxidants	Root	1.87± 0.12	1.92± 0.18
	Stem	2.12± 0.09	2.19± 0.07
	Leaves	1.86± 0.12	1.69± 0.18*
Flavonoids	Root	0.09± 0.01	0.11±0.01
	Stem	0.13 ±0.01	0.14 ± 0.01
	Leaves	0.07 ± 0.01	0.08 ± 0.01
Phenolics	Root	7.08±0.82	7.53± 0.19*
	Stem	6.59± 0.82	6.74± 0.18
	Leaves	5.16 ± 0.54	6.07± 0.45**
Total proteins	Root	1.38± 0.14	1.56± 0.01*
	Stem	2.05 ± 0.12	1.78 ± 0.10*
	Leaves	1.76 ± 0.03	1.72 ± 0.21
Total Sugars	Root	169.68± 0.52	99.22± 0.11**
	Stem	167.62± 0.12	167.67± 0.17
	Leaves	153.68± 3.06	119.22± 0.11**
Reducing sugars	Root	1.96±0.01	1.71± 0.06*
	Stem	1.48± 0.04	1.66± 0.01*
	Leaves	1.52 ± 0.01	1.41± 0.06

*Students T-Test used for comparison between two means of each variable taking P-value ≤ 0.05 as significant, * Significant, ** Highly Significant.*

Antimicrobial activity from different parts of *C. sinensis*: Plants have great potential to synthesize different bioactive compounds to protect themselves versus different plant pathogenic microbes and insects. These bioactive compounds are used since ancient time as folk medicine and beneficial for human being to relieve different pathogenic diseases.

In the present investigation, *in-vitro* antimicrobial activity from acetone and water extracts of numerous segments of *C. sinensis* plant such as roots, stems and leaves were tested (Table-3). The antibacterial activity was also carried out in the same extracts against *Staphylococcus aureus*. The maximum activity was noted in stems acetone extract (30.2 ± 1.53 mm) followed by stems water extract (28 ± 2.10 mm), roots acetone extract (25 ± 0.15 mm), roots water extract (22 ± 02.53 mm), leaves acetone extract (14 ± 1.21 mm) and leaves water extract (15.25 ± 0.40 mm).

On the other hand, towards *Pseudomonas aeruginosa*, the maximum activity was noted in stems acetone extract (28.15 ± 0.05 mm) as compared to stems water extract (26 ± 0.53 mm), roots acetone extract (5.3 ± 0.23 mm), roots water extract (23 ± 0.12 mm), leaves acetone extract (20 ± 1.21 mm) and leaves water extract (19.75 ± 0.50 mm) respectively. In addition, *Klebsiella pneumonia* was applied to observe the antibacterial activity of *C. sinensis*. The higher activity was recorded in stems acetone extract (26.5 ± 0.70 mm) but the growth inhibition measured in comparatively low in other parts of under study plant as stems water extract (24.3 ± 1.31mm), roots acetone extract (21 ± 0.80 mm), roots water extract (20 ± 0.01 mm), leaves acetone extract (18.5 ± 0.80 mm) and leaves water extract (18 ± 0.90 mm) correspondingly as shown in Table- 3. The present results exhibited that different parts of *C. sinensis* extracts ceased the growth of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. It was noticed that the presence of bioactive compounds such as phenolics, saponins, alkaloids and flavonoids etc. in all extracts may cause the inhibition of bacterial species.

Table 3. Antibacterial activity of *C. sinensis*' segments extracts against different bacterial species.

Bacterial Species	**Plant's segments**	Acetone extract (mm)	**WaterExtract(mm)**
Staphylococcus aureus	Leaves	14 ± 1.21	15.25± 0.40*
	Stems	30.2 ± 1.53	28 ± 2.10
	Roots	25 ± 0.15	22 ± 02.53*
Psedeumonas aeronginosa	Leaves	20 ± 1.21	19.75 ± 0.50
	Stems	28.15 ±0.05	26 ± 0.53*
	Roots	25.3 ± 0.23	23 ± 0.12*
Klesibella pneumonia	Leaves	18.5 ± 0.80	18 ± 0.90
	Stems	26.5 ± 0.70	24.3±1.31*
	Roots	21 ± 0.80	20 ± 0.01

Students T-Test used for comparison between two means of each variable taking P-value ≤ 0.05 as significant, * Significant, ** Highly Significant.

The strong antibacterial effects of the acetone extract against the three types of bacteria *Staphylococcus* aureus (30.2 ± 1.53 mm), *Pseudomonas aeruginosa* (28.15 ± 0.05 mm) and *Klebsiella pneumonia* (26.5 ± 0.70 mm) as shown in Table-3. It was proved that acetone and water extracts of plant *C. sinensis'* segments have different chemical compositions, hence showed varying degrees of antibacterial potential. In general acetone extracts ceased bacterial growth more efficiently in comparison to water extracts. In addition, the lethal efficacy of some standard antibiotics were also checked against three species of bacteria. Piperacillin / Tazobactam showed negative (–ve), 5 mm and 5 mm towards *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia.* Avelox revealed 12 mm, negative (-ve) and 20.5 mm against S. *aureus, Pseudomonas aeruginosa* and *K. pneumonia.* Oxacillin displayed negative (–ve) result towards S.*aureus, Pseudomonas* and 5 mm for *K. pneumonia.* Erythromycin presented highest antibacterial activity for all bacteria 15, 18 and 20 mm respectively. Ofloxacin showed 14.8 against *Staphylococcus aureus* and negative for *Pseudomonas aeruginosa.* Furthermore, pure acetone (without aqueous dilution) was applied in well as a control to check the antimicrobial activity but no any antimicrobial activity was noted which proved that acetone evaporated during inoculation.

Antifungal activity of different extracts of *C. sinensis:* The inhibition zone diameter obtained by different parts of *C. sinensis* extract that revealed acetone extracts of leaves, stems and roots had the highest zone of inhibition as compared to the water extracts (Table-4). The maximum zones of inhibition were measured against *Aspergillus niger* in stems acetone extract (35.5 ± 0.70 mm) followed by stems water extract (30.5 ± 0.70 mm), roots acetone extract (29 ± 1.2 mm), roots water extract (27 ± 2.82 mm), leaves acetone extract (25 ± 1.41 mm) and leaves water extract (24.5 ± 0.35 mm). The highest zones of inhibition were measured against *Candida albicans* in stems acetone extract (29.5 ± 0.70 mm) followed by stems water extract (28.5 ± 0.2 mm), roots acetone extract (25.5 ± 0.7mm), roots water extract (24.5 ± 0.12 mm), leaves acetone extract (19.7 ± 0.3 mm) and leaves water extract (22.35 ± 0.05 mm).

The highest zones of inhibition were observed against *Penicillium notatum* in roots acetone extract (25.7 ± 0.7 mm) followed by stems acetone extract (24.5 ± 0.05 mm), roots water extract (23.5 ± 0.05 mm), stems water extract (22.75 ± 0.32 mm), leaves water extract (19.5 ± 0.9 mm) and leaves acetone extract (15 ± 0.3 mm).

Table 4. Antifungal activity from extracts of *C. sinensis* against different fungal species

Fungal Species	Plant's segments	Acetone Extract(mm)	Water Extract (mm)
Aspergillus niger	Leaves	25 ± 1.41	24.5 ± 0.35*
	Stems	35.5 ± 0.70	30.5 ± 0.70**
	Roots	29 ± 0.0	27 ± 2.82*

Candida albicans	Leaves	19.7 ± 0.3	22.35 ± 0.05*
	Stems	29.5 ± 0.70	28.5 ± 0.2*
	Roots	25.5 ± 0.7	24.5 ± 0 .12*
Penicillium notatum	Leaves	15 ± 0.3	19.5 ± 0.9**
	Stems	24.5 ± 0.05	22.75 ± 0.32*
	Roots	25.7 ± 0.7	23.5 ± 0.1*

Students T-Test used for comparison between two means of each variable taking P-value ≤0.05 as significant, * Significant, ** Highly Significant.

Discussion

Phytochemical screening is a qualitative chemical examination method of secondary metabolites contained in plant extracts. Moreover, screening is the initial step to verify the presence or absence of certain chemical compounds in plant that can be related with biological activity.

It is documented that these secondary metabolites have numerous biological and diseases curing properties (Benedec *et al.*, 2013, Proestos *et al*., 2013, Rao *et al*., 2012 and Vishnu *et al*., 2013). Tannins are considered major components of secondary metabolites which have several properties such as anti-diarrhea, astringent, antioxidant and antibacterial. Generally, tannins are recognized as polyphenol compounds having high molecular weight and have ability to form complexes with protein (Yildirim and Kutlu 2015). Eltayeib and Ishag (Eltayeib and Ishag 2015) have reported that methanol extract of *Cordia sinensis* bark contains a high amount of tannin. It was also investigated by Abaka *et al*., (2020) that extraction of *Balanites aegyptiaca* Callus and seeds using methanol/n-hexane solvent mixture contains a low amount of tannin. Owolabi *et al.*, (2018) have used diethyl ether, ethanol and water extract of *Feretia apodanthera* root bark which contain tannin. A small amount of tannin was also reported (Roghini and Vijayalakshmi 2018) in ethanol and water extract of *Citrus paradise*. Muhongo *et al*., (2021) have prepared different solvents and water extracts of *Pechuel-Loeschea leubnitziae* leaves and found that hexane, ethyl acetate, ethanol and methanol extracts contained tannin but were absent in aqueous extract.

Terpenoids belong to natural products in nature derived from isoprene units. Terpenoids have great impact on human health including anticancer, anti-inflammation, antiviral and other functions (Bohm *et al.*, 2020 and Hill and Connolly 2020). Owing to the good antiviral effectssuch as SARS-CoV-2 and hepatitis C virus (Chao *et al.*, 2016), several terpenoids have been applied in treatment of COVID-19 (Bailly and Vergoten 2020).

The similar pattern of results was also observed (Roghini and Vijayalakshmi 2018) by using ethanol, water, ethyl acetate and n-hexane extract of *Citrus paradise* and found all these extracts contain terpenoid. Similar findings have also been observed by Muhongo *et al.*, (2021) toprepare hexane, ethyl acetate, ethanol, methanol and aqueous extract of *Pechuel-Loeschea leubnitziae* leaves and found that all extracts contain terpenoid.

In nature, flavonoids are the biggest group of phenol compounds. Flavonoids are frequently found in plants as glycosides and are found in all parts of the plant together with pollen, fruit, and roots (Phull *et al*., 2020). For the confirmation of flavonoids, each part of the plant was analyzed through the addition of chemicals in the extracts. The appearance of yellow precipitates showed the presence of flavonoids in plant extracts. The present study results of flavonoids were also compared with the results obtained by several researchers by using different plant extracts. (Khan *et al.*, 2021) have reported that the leaves sample exhibited the highest flavonoids as compared to methanolic extract of roots, stems bark and seeds of *A. nitida*. It was also described by Muhongo *et al.*, (2021) that only ethanol, methanol and aqueous leaf extracts of *Pechuel-Loeschea leubnitziae* contain flavonoids while absent in hexane and ethyl acetate extract. (Owolabi *et al.*, 2018) have reported that all three extracts (diethyl ether, ethanol and water) of *Feretia apodanthera* root bark possess flavonoids. Furthermore Roghini and Vijayalakshmi (2018) have also investigated flavonoid content from different solvent extracts of *Citrus paradise* and found that the ethanol extract contains a moderate amount of flavonoids, ethyl acetate and water extract small amount while absent in n-hexane extract.

Alkaloids are the largest group of naturally occurring organic compounds of plant secondary metabolites (Rosales *et al*., 2020). Beside dietary uses, alkaloids have also a major role in human medical history and are broadly applied for the treatment of several diseases such as cancer (Dey *et al.*, 2019), neurological disorders (Hussain *et al.*, 2018), metabolic disorder (Feng *et al.*, 2019) and could be used for the treatment of COVID-19 treatment (Majnooni *et al.*, 2021). The present under study plant's segments exhibited the presence of alkaloids. Our findings are the good agreement of other workers of relevant field. (Batubara *et al*., 2020) have foundthat the alkaloid compounds were present in the ethanol extract of agarwood leaves collected from Hutanabolon Village, but was absent in the agarwood leaves collected from Laru village, while Eltayeib and Ishag (2015) have revealed that petroleum ether extract of *C. sinensis* bark contains good amount while methyl trichloride and ethyl acetate extract contain low amount of alkaloids. On the other hand, Owolabi *et al.*, (2018) have found in other

plants species that only ethanol extract of *Feretia apodanthera* root bark contains alkaloids while absent in diethyl ether, n-hexane and water extracts. It was also revealed (Gul *et al*., 2017) that both the methanolic and ethanolic extract of *the Ephedra intermedia* plant contain alkaloids.

Several researchers have investigated and documented the presence of saponins in different plants and solvents extraction such as (Abaka *et al.*, 2020) have prepared extracts of *Balanites aegyptiaca* Callus using methanol n-hexane solvent mixture and found that this extract contains a moderate amount of saponins. Owolabi *et al.*, (2018) have prepared ethanol and aqueous extract of *Feretia apodanthera* root bark and detected saponins content. Roghini and Vijayalakshmi(2018) have revealed that ethanol and water extract of *Citrus paradise* contains a small number of saponins, while absent in n-hexane and ethyl acetate extract. On the other hand, only hexane and ethanol extracts of *Pechuel-Loeschea leubnitziae* leaves contain saponins while absents in methanol, ethyl acetate and water extracts (Muhongo *et al.*, 2021).

In the human body antioxidants are effective versus some metabolic syndromes such as protein and lipidic peroxidation, cell membrane alteration and DNA degradation (Miron *et al.*, 2019). It is well documented that various medicinal plants have high antioxidant activity (Zivkovic *et al*., 2012). The results shown in Fig.1, indicated that *C. sinensis* is a rich source of antioxidants. Senguttuvan *et al*., (2014) have reported that the highest antioxidant activity was exposed by leaves methanol extract of *Hypochaeris radicat* (90.43%) and roots aqueous extracts (90.34%) and leaves water extract near to 80%. Benmaghnia *et al.*, (2021) have extracted three samples of Dried figs collected from different places and found that acetone extract contained 54.8, 50.5 and 51.5 mg AAC / g DM respectively while aqueous extract of other places contained 93.8, 53.9 and 71.2 mg AAC / g DM correspondingly.

The available literature illustrates no conclusive work showing the correlation between antioxidant capability and phenolic content (Konan *et al*., 2014 and Ruberto *et al.*, 2007) but some researchers have found a strong relationship between both antioxidant and phenolics (Makris *et al*., 2007 and Sankhalkar and Vernekar 2016). In the current investigation the significantly higher quantity of phenolic content in *C. sinensis* Lam. is also correlated with increased antioxidant activity. Our results are higher than some other plant species' extracts such as Sankhalkar and Vernekar (2016) have revealed that the total phenolic content in methanolic leaves extract of *Moringa oleifera* was 2.28 ± 0.022 mg/mL and in flower 1.08 ± 0.0025mg/mL while methanolic leaves extract of *Ocimum tenuiflorum* L 2.18 ± 0.015 mg/mL and in flower 1.73 ± 0.0015 mg/ml. Low amount of total phenolic content has also been investigated in methanolic crude extracts of *Thymus vulgaris* (Hossain *at el*., 2013). Same pattern of results was also observed by (Bruck de Souza *et al.*, 2020) when used methanolic extract of leaves of *Chaptalia nutans*. Senguttuvan *et al.*, (2014) have investigated various extracts of roots and leaves of *H. radicata* and estimated total phenolics which was found variable broadly between 0.32 to 5.04 mg GAE/100 g extract but noticed that methanolic extract of leaves and roots parts were showing higher total phenolic content (3.75 and 5.04 mg GAE/100 g extracts correspondingly) and water extract contained 0.32 and 0.70 mg GAE/100 g extracts respectively as compared to other tested solvents extracts. Duru (2021) has prepared ethanolic extract of Brown, Green and Red *Propolis* and found that phenols content 29.11, 14.68 and 46.99 µg/g respectively. On the other hand some researchers have obtained quite higher amount of total phenol content from different plants species and solvent extracts such as Johari and Khong (2019) both observed that methanolic extract of *P. bleo* contained phenolic compounds 40.82 mg GAE/g. Graziela *et al.*, (2021) have estimated phenolic content in hydroalcoholic extract of *Plinia peruviana* leaves and found 944 ± 0.0856 mg GAE/g. Muhongo *et al.*, (2021) have prepared *Pechuel-Loeschea leubnitziae* leaves extract in methanol and water and estimated total phenol in methanol 58.70 ± 0.21 and water 57.14 ± 0.05 µg GAE/mg respectively. It was well revealed that phenols and phenolic extracts contain antimicrobial activity which make them favorable alternatives to chemical preservatives and antibiotics (Duru 2021).

The quantity flavonoids contents were quite low in our findings than the findings of others like Khan *et al.*, (2021) have observed the maximum flavonoid concentration (53.25 mg QE/g) in the leaves extracts of *A. nitida*. High flavonoids content (21.64 mg/g) were found in methanolic extract of leaves of *Chaptalia nutans* (Bruck de Souza *et al*., 2020). The concentration of total flavonoids was highest in ethyl acetate leaves extract (17.79 mg RE/100 g extract) followed by chloroform and ethyl acetate roots extracts (14.31 and 14.28 mg RE/100 g extract respectively and low in water extract 6.68 and 3.61mg RE/100 g extract respectively (Senguttuvan *et al.*, 2014). Ethanolic extract 3.83 ± 0.05, water extract 1.88 ± 0.01 and ethyl acetate extract 1.11± 0.05 mg/g was also reported by Roghini and Vijayalakshmi (2018). Graziela *et al.*, (2021) have reported that the hydroalcoholic extract of *Plinia peruviana* leaves contain 531.8 ± 0.0040 mg RE/g flavonoid content whil Muhongo *et al.*, (2021) have prepared extract of *Pechuel-Loeschea leubnitziae* leaves in ethanol contains 47.82 ± 0.29, methanol 78.84 ± 0.47 and water 30.41 ± 0.12 µg /mg flavonoid respectively. It was reported that the acetone is the best solvent according to the results representing the level of flavonoids, polyphenols and tannins which was accepted by earlier reports (Al-Farsi and Lee 2008). It is well known that usage of solvent for extraction and isolation of bioactive molecules is very effective (Agbafor *et al*.,

2015).

The present results exhibited that different parts of *C. sinensis* extracts ceased the growth of *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. It was noticed that presence of bioactive compounds such as saponins, alkaloids and flavonoids etc. in all extracts may cause the inhibition of bacterial species.

This is in harmony with reports of Jaberian *et al*., (2013), that the presence of effective bioactive compounds such as alkaloids, flavonoids and tannins etc. in plant extracts may cause the antibacterial activities. The strong antibacterial effects of the acetone extract against the three types of bacteria *Staphylococcus* (30.2 ± 1.53 mm), *Pseudomonas* (28.15 ± 0.05 mm) and *Klebsiella pneumonia* (26.5 ± 0.70 mm) as shown in Table-3. It was proved that acetone and water extracts of plant *C. sinensis'* segments have different chemical compositions, hence showed varying degrees of antibacterial potential. In general acetone extracts ceased bacterial growth more efficiently in comparison to water extracts.

The present results findings revealed that acetone extract could be useful in controlling the development of tested fungal. It was also observed that *the C. sinensis* plant displayed not only effective antibacterial agent as well as various segments of under studied plant also strongly ceased the growth of three tested fungal species *Aspergillus niger*, *Candida albicans* and *Penicillium notatum* and found the best inhibitor against the fungus growth or as a best antifungal agent. The excellent antimicrobial activity in plants may be relevant to the occurrence of phytochemicals including flavonoids, saponins, tannins, alkaloids and phenolic acids (Jeong *et al*.,2009, Jouda *et al*., 2015and Rashid *et al*., 2014).

Conclusions

From the findings of the study, it may be concluded that the acetone extract of *C. sinensis* acts as the potential source of bioactive compounds that may be used in traditional medicine for the prevention of several diseases. Therefore, *C. sinensis* can assist as a good source of therapeutic compounds and treatment of bacterial and fungal relevant diseases. However, there is further need of investigation in terms of suitable methods for effective isolation of bioactive compounds through reliable analytical techniques and clinical trials for their effectivenessin bacterial and fungal relevant diseases are future research perspectives.

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Conflicts of Interest

The authors declare no conflict of interest.

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