PHYTOCHEMICAL, ANTIBACTERIAL, AND CYTOTOXICITY ANALYSES OF REDFLOWER RAGLEAF (*Crassocephalum crepidioides*) LEAVES LIQUID EXTRACT

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ABSTRACT *– The Redflower Ragleaf (Crassocephalum crepidioides) is a plant species indigenous to tropical Africa and of cultural and medicinal significance. In this study, the phytochemical, antibacterial, and cytotoxicity potentials of liquid extract from the leaves of Redflower Ragleaf (C. crepidioides) were investigated. Quantitative research with experimental design assessed the phytochemical activity through Wagner, Ferric chloride, and Salkowski tests on alkaloids, tannins, and terpenoids, respectively. The study utilized the Kirby-Bauer technique for the antibacterial activity of Escherichia coli and Staphylococcus aureus, while cytotoxicity analysis was performed using the Brine Shrimp Lethality Assay. The result showed that the three secondary metabolites in the plant were reddish-brown in alkaloids, greenish-black in tannins, and red-black and dark green in terpenoids. However, the four predetermined concentrations (0 ppm, 200 ppm, 500 ppm, and 1000 ppm) of C. crepidioides liquid extract did not demonstrate antibacterial efficacy against the tested bacteria, indicating their resistance to the treatment. The positive control showed susceptibility to the treatment with a diameter of 17.0 mm for E. coli and 31.9 mm for S. aureus, within the range of ≥ 13 - ≥ 29 mm for susceptibility interpretation. Meanwhile, cytotoxicity analysis showed no toxicity level based on brine shrimp mortality rates, with an LC_{50} of 79,432.82 ppm. The six concentrated extracts (0 ppm, 75 ppm, 200 ppm, 500 ppm, 750 ppm, and 1000 ppm) were considered non-toxic based on Meyer and Clarkson's toxicity criteria. To expand comprehension of C. crepidioides, further research on other parts of the plant, utilizing different secondary metabolites, studying different bacteria strains, and performing varied extraction methods and solvents are recommended. Additionally, alternative toxicity assays and larger specimen populations should be considered.*

Keywords – *Antibacterial, Brine Shrimp Lethality Assay, Crassocephalum crepidioides, Cytotoxicity, Kirby-Bauer technique, Phytochemical, Redflower Ragleaf*

1. INTRODUCTION

In the Philippines, Filipinos have adopted a profound relationship with nature's bounty, drawing from a wealth of medicinal plants that are deeply ingrained in their culture and traditional medical procedures. The Redflower Ragleaf (*Crassocephalum crepidioides*) plant, also known as *ebolo*, thickhead, or *sapsapon*, has been classified as part of the *Asteraceae* (sunflower family) [1]. *C. crepidioides* is a moderately invasive herb, especially in tropical and subtropical areas. It is a pioneer species that disperses seeds by wind, and its distribution in China may be linked to cultivation [2]. This plant is versatile and can be used as a weed, vegetable, and animal feed containing various essential minerals and proliferates, yielding a large crop, especially in gray soil. It has long been used for therapeutic purposes [3].

Research that particularly examines the significance of the herbal medicine Redflower Ragleaf is lacking despite the growing interest in using natural plants for therapy and prevention and the widespread occurrence of said plants in the community [4]. Phytochemical screening does not only reveal the constituents of plant extracts, specifically *C. crepidioides*, by which one predominates over others, but it also aids in the search for bioactive agents that can be used in the synthesis of valuable drugs [5]. By discovering the plant's different phytochemical components, one can use it as a basis to determine its valuable and usable natural source of medicine not just in one community but also in modern medicine [6].

The rising worldwide antibiotic resistance crisis offers a significant challenge to modern medicine, emphasizing the critical need for new antibacterial remedies. With the shortage of antibiotic resistance and the decreasing efficacy of traditional antibiotics, there is an urgent need to investigate alternate sources of antibacterial agents, particularly those derived from natural substances [7]. Bacterial pathogens are one of the most significant issues in the medicinal world since they have developed antibacterial resistance and evolved diverse strategies for colonizing and invading human organs [8]. Accordingly, one of the good sources of antibacterial agents is medicinal herbs [9]. However, no evidence supports that medicinal herbs, specifically *C. crepidioides* on *Escherichia coli* and *Staphylococcus aureus*, exhibit antibacterial properties. Also, before natural plant products can be used as antimicrobial agents, their cytotoxicity must be assessed to ensure human safety [10].

In addition, *C. crepidioides*, with its traditional medicinal properties, offers possible options for investigations as it has been noted to have hypoglycaemic, antioxidant, and anti-inflammatory properties [11]. Also, this plant contains bioactive components with anticoagulant characteristics that could be used to treat blood coagulation problems [12]. Moreover, traditional medicine in China, India, and Russia have collectively acknowledged the hepatoprotective attributes *of C. crepidioides* for its liver tonic action. This plant's thick, mucilaginous leaves and stems are vegetables, while fresh green shoots are cooked or boiled for soup [13]. Eating *C. crepidioides* may aid in preventing and managing heart disease, cancer, and diabetes. It may also help in preventing blood clots and have anti-inflammatory and anti-tumor properties [14].

Furthermore, identified phenolic compounds of this plant include antioxidant and acetylcholinesterase inhibitory potential. The ethanol extract inhibited the enzymatic activity of 5-lipoxygenase [15]. Meanwhile, secondary metabolites profiling and antioxidant activity of *C. crepidioides* reveal the presence of compounds with potential medicinal benefits [16]. Moreover, phytochemicals are secondary plant metabolites that are naturally occurring biologically active plant compounds with potential disease-inhibiting capabilities that *C. crepidioides* may already acquire. It is also believed that phytochemicals may effectively combat or prevent diseases due to their antioxidant effect [17]. *C. crepidioides* is also used in traditional African medicine to treat indigestion, stomachache, epilepsy, sleeping sickness, and swollen lips [2]. Its great potential for medicine made the community interested in using it as a medicinal alternative for various diseases [18].

This study aimed to comprehensively aid the discovery of new sources of medication by discovering phytochemical components of *C. crepidioides* leaves liquid extract in terms of alkaloids, tannins, and terpenoids that may be applied to modern medicine. Also, this study aimed to examine whether the leaves' liquid extract of Redflower Ragleaf (*C. crepidioides*) exhibits antibacterial activity against *E. coli* and *S. aureus*. Additionally, it sought to assess the safety profile of *C. crepidioides* through the Brine Shrimp Lethality Assay, providing insights into its potential toxicity. By achieving these objectives, the findings of this study not only supported the traditional therapeutic benefits associated with the plant but also contributed vital scientific understanding that guided its safe and successful medicinal utilization and its potential as a natural antibacterial agent.

2. MATERIALS AND METHOD

Research Design

This study utilized quantitative research, specifically experimental design. This study chose the zone of inhibition and mortality rate as the dependent variables. The independent variable was the concentrations of liquid extract from *C. crepidioides* and the positive control. By utilizing this research design, the researchers comprehensively identify the secondary metabolites present, such as *C. crepidioides*, and their antibacterial and cytotoxicity potentials.

Entry Protocol

Before conducting the study, the researchers submitted an official letter of request to the Office of the School Principal, asking for authorization to conduct the study. To ensure ethical information gathering, researchers also gathered consent for a permit secured from the locality, including the landowner. Furthermore, the Office of Forest and Welfare Research, Development, and Extension Center (FWRDEC), located in Region 10, Station Office, Sumpong, Malaybalay City, Bukidnon, also received a letter asking for further identification of the samples that were collected. Moreover, another letter was sent to the Office of the Central Mindanao University-National Product Research and Development Center (NPRDC) Laboratory and College of Veterinary Medicine, indicating that they were involved in this study for the processes of the samples and antibacterial analysis, respectively. Finally, the parents of participating students were asked to sign a consent form that entails their agreement to release the institution from any obligation for any bad experiences their children had while participating in the study off-campus.

Locale of the Study

The collection of samples was conducted in P-2 Guinuyoran, Valencia City, Bukidnon, and delivered to the Office of Forest and Welfare Research, Development, and Extension Center (FWRDEC), located in Region 10, Station Office, Sumpong, Malaybalay City, Bukidnon for final identification. Institutions such as San Isidro College, Malaybalay City, Bukidnon, and Central Mindanao University, Maramag, Bukidnon, were also involved in the processes and analysis of the study.

Collection and Preparation of Samples

The researchers started by collecting samples of Redflower Ragleaf (*C. crepidioides*) from P-2 Guinuyoran, Valencia City, Bukidnon. The samples were adequately washed with distilled water and were sun and air-dried for 14 days at room temperature. The plant's leaves were cut into small pieces and pulverized using an electric blender. Afterward, the powdered samples were stored in an airtight container, as stated in [19].

Identification of the Redflower Ragleaf Specimen

The specimen was brought to the Forest and Welfare Research, Development, and Extension Center (FWRDEC), located in Region 10, Station Office, Sumpong, Malaybalay City, Bukidnon, for final identification. The identification process was thorough, examining each sample's macroscopic and microscopic attributes. Proper identification and classification of the plant species was done in consultation with the botany experts, ensuring that every sample was accurately identified.

Extraction Process

For phytochemical screening, 30 g of powdered samples were soaked up with 300 mL of 95% ethyl alcohol for five days and then filtered out using a filter paper. To avoid contamination, the extract was stored in a container with a volume of 10 mL to test for the phytochemical components and to perform and examine its secondary metabolite [20]. The powdered samples were brought to the Central Mindanao University Natural Product Research and Development Center (NPRDC) Laboratory for antibacterial and cytotoxicity analysis to undergo maceration, rotary evaporation, and reconstitution processes. The samples were macerated to support the study with slight modifications [21]. The finished 500 g powdered samples were steeped in 95% ethanol, leaving 1 inch above the sample, for 72 hours at a temperature of 4° C. After filtering the ethanol-based extract via filter paper, it was collected in a flask, and its weight was recorded to undergo rotavap. The rotary evaporation process was congruent with another study's process where a flask with a rounded bottom was submerged in a water bath, and the flask was rotated while partially evacuated. As a result of the apparatus's decreased pressure, the solvent boils at a lower temperature, which guarantees the flask's safe attachment to the bump trap [22]. After the rotavap, the extract was reconstituted with 5% Dimethyl sulfoxide (DMSO) at 4° C under reduced pressure to get the liquid extract of the sample and kept in the vial [23]. For the

following experiments, this solution was further diluted to different concentrations. For antibacterial analysis, the extract was diluted into four different concentrations with volumes of 4 mL. For cytotoxicity analysis, the extract was diluted into six different concentrations with volumes of 25 mL.

Preparation and Implementation of Phytochemical Analysis

The following were the different tests that this study utilized to identify the secondary metabolite of *C. crepidioides* leaf extract following the process of [24].

Test for Alkaloids

The 2 mL of dilute hydrochloric (HCl) acid was added to 2 mL of the ethanolic sample extract. After that, a drop of Wagner's reagent was then applied. The presence of a reddish-brown color confirmed the test was positive.

Test for Tannins

The ferric chloride test was used to determine the presence of Tannins in the leaf extract—1 mL of 5% ferric chloride. The formation of dark blue and greenish-black indicated the presence of tannins.

Test for Terpenoids

The Salkowski test was used to determine the presence of Terpenes in the leaf extract. 2 mL of chloroform and 2 mL of sulphuric acid were added to 0.5 mL of the ethanolic extract. The formation of a red-brown color showed the presence of Terpenoids.

Preparation and Implementation of Antibacterial Analysis

Bacteria *E. coli* and *S. aureus* are the most suitable bacteria for educational settings due to their known zone sizes [25]. In addition, this analysis was performed according to the method of [26] with minor modifications. The antibacterial analysis process involved soaking filter paper discs in extract for 24 hours and preparing a bacterial suspension. The suspension was then inoculated onto Mueller-Hinton agar using the Kirby-Bauer technique. The bacterial suspension was then plotted in Mueller Hinton Agar with the antibiotic disc as a positive control, ensuring each disc had enough space to avoid overlapping inhibition zones. Furthermore, the Mueller Hinton Agar plate was inverted at 37 °C for 24 hours, and the zone of inhibition of each antibiotic or filter paper disc was read and recorded. This method ensures that the bacteria is effectively controlled and used in educational settings.

Preparation and Implementation of Cytotoxicity Analysis

For this process, a method with minor modifications was utilized [27]. This study involved preparing a brine shrimp hatchery tool set, filling a container with 250 mL of tap water, and adding one teaspoon of rock salt and a pinch of baking soda to create artificial seawater. A half teaspoon of Ocean Star International (OSI) brine shrimp eggs were placed in the incubator. In 72 hours, the *nauplii* were harvested and placed in a vial for cytotoxicity testing. Six concentrations (0 ppm, 75 ppm, 200 ppm, 500 ppm, 750 ppm, and 1000 ppm) were replicated five times with three trials per replication. Two drops of brine shrimps and 100 µl of substitute seawater were placed in a 96-well microplate for 24 hours and labeled accordingly. The 96-well microplates were used to determine the medium lethal concentration (LC_{50}) of various soluble products in saltwater, whether artificial or natural, in a straightforward, affordable, and quick manner [28] [29]. To continue, after a 24-hour incubation period, 100 µl of each concentrated extract was introduced into the wells and allowed to settle for 15 minutes. Using a microscope, the brine shrimp were observed and counted to differentiate between alive and dead brine shrimps.

Data Gathering Procedure

Secondary metabolite analysis, specifically alkaloids, tannins, and terpenoids, was discussed. Wagner's test was used to determine alkaloids. A ferric chloride test was utilized to determine tannins. The Salkowski test was used to analyze the terpenoids. The data-gathering procedure for antibacterial analysis included disk diffusion using the Kirby-Bauer method in an antibacterial test as part of the data collection process. This process required filter paper, distilled water, Mueller-Hinton agar, and petri dishes. Meanwhile, cytotoxicity analysis was done through the Brine Shrimp Lethality Assay (BSLA). This analysis acquired certain materials and equipment, such as OSI brine shrimp eggs with a 90% hatching rate, tap water, seawater, a plastic dropper, filter paper, 96 wells microplate, baking soda, vials, thermometer, a microscope, and a brine shrimp hatchery tool set.

Data Analysis Procedure

For the phytochemical test, the study utilized Wagner, Ferric chloride, and Salkowski tests on alkaloids, tannins, and terpenoids, which helped this study to determine comprehensively whether this plant has these compounds. For the antibacterial test, the zone inhibition was measured using the Zone of Inhibition Testing or Kirby Bauer's Test, which helped this study to measure the potential of liquid extract in preventing the growth of the chosen bacteria. The diameter was measured using a caliper on the underside of the petri dish. On the other hand, the results from the cytotoxicity analysis were recorded and interpreted using mortality rate, linear regression, and LC_{50}, which helped this study identify whether the plant's leaves had a cytotoxicity level between the mean of the dead brine shrimp *nauplii* after treating with various concentrations of the liquid extract. Also, linear regression was used to determine what concentration has the highest and lowest mean mortality rates [30].

Statistical Analysis

The data gathered from the test was carefully recorded, analyzed, and interpreted accordingly based on the statistical analysis results. The researchers employed descriptive statistics such as percentage and mean.

Ethical Consideration

The researchers handled the data with respect and care to ensure its integrity and dependability. Safety precautions were also taken. Furthermore, the school provided the necessary tools and resources for the investigation, so the researchers were responsible for their actions and adhered to the study's guidelines and rules.

Documentation

The researchers photographed the Redflower Ragleaf (*C. crepidioides*) species from their natural environment. The photos were taken to document the plants' natural state. These samples were used for future reference and to share information about the plant with others.

3. RESULTS AND DISCUSSION

Phytochemical Analysis

Secondary metabolism is a precise regulator of plant growth and development, which serves as a reserve of essential phytochemicals and shields plants from various environmental constraints [31]. Plant defense against herbivory and other interspecies frequently depends heavily on secondary metabolites. Humans use secondary metabolites as medicines, flavorings, pigments, and recreational drugs. It also created safe and effective medication as a single compound or combination [32]. These metabolites give plants the ability to quickly recognize herbivore attacks and act rapidly in a pest and environment that is constantly changing. Furthermore, beneficial natural substances are produced by a plant's metabolites, according to [33].

Table 1 Alkaloids, Tannins, and Terpenoids Test on *C. crepidioides* Ethanolic Extract

Phytochemicals and Tests used	Trials	Results	Remarks
Alkaloids using Wagner Test	1	Reddish-brown	Positive
	2	Reddish-brown	Positive
	3	Reddish-brown	Positive
	4	Reddish-brown	Positive
	5	Reddish-brown	Positive
Tannins using Ferric chloride Test	1	Greenish-black	Positive
	2	Greenish-black	Positive
	3	Greenish-black	Positive
	4	Greenish-black	Positive
	5	Greenish-black	Positive
Terpenoids using Salkowski Test	1	Red-brown	Positive
	2	Red-brown	Positive
	3	Dark green	Negative
	4	Dark green	Negative
	5	Red-brown	Positive

Table 1 shows the phytochemical tests with five trials for each test. The testing of alkaloids exhibited a reddish-brown color, which indicates in the table that the *C. crepidioides* was positive for the presence of an alkaloid. It may be due to particular toxins that help produce antimicrobials, deter herbivores, and protect the plant from being eaten. *C. crepidioides* has pyrrolizidine alkaloids (PAs) that are heterocyclic secondary metabolites with a typical pyrrolizidine motif predominantly produced by plants as defense chemicals against herbivores. They display a wide structural diversity and occur in many species, continuously discovering novel structures and occurrences [34].

Moreover, the tannins tested had a greenish-black color, indicating that *C. crepidioides* was positive for the presence of this secondary metabolite. This result may be because tannins are phenolic compounds with large molecular weights ranging from 500 Da to more than 3000 Da, which they discovered in plant leaves, bark, fruit, wood, and roots, primarily in the tissues in the vacuoles. They are linked to plant defense mechanisms against mammalian herbivores, birds, and insects and to protecting plants from infections [35].

Furthermore, for the testing of terpenoids, the table indicated that trials 3 and 4 resulted in a dark green color that appeared to be negative for terpenoid testing. Trials 1, 2, and 5 are positive, indicating a red-brown color. Since the Salkowski test in positive trials is more significant than in negative trials, this study considers and concludes that the Redflower Ragleaf (*C. crepidioides*) result is positive in terpenoids phytochemical testing. That being the case, the result may be because the plant *C. crepidioides* is a defense against biotic and abiotic stresses, or they are treated as signal molecules to attract insects to pollination. In addition, terpenoids are chemical molecules found in all living creatures, such as green plants, mainly flowering plants, with high-quality terpenoids [36]. PA-containing plants are used in herbal medicines in many countries, and their beneficial biological activities to cure disease have drawn greater attention [37].

Antibacterial Analysis

The global public health community has been increasingly concerned about antibacterial resistance, prompting the quest for substitute antimicrobial drugs [38]. The inhibitory zone diameter of *C. crepidioides* leaves liquid extract concentrations and treatment on *E. coli* and *S. aureus* was summarized, recorded, and measured in Table 1 below.

Table 2. Zone of Inhibition Using Various Treatments and Concentrations of *C. crepidioides* Leaves Liquid Extract

Antibiotic/Extract	Bacteria/Isolate	Zone of inhibition (mm)			Interpretation
		R1	R2	R3	
(0 ppm)	*S. aureus*	0	0	0	Resistant
	E. coli	0	0	0	Resistant
(200 ppm)	*S. aureus*	0	0	0	Resistant
	E. coli	0	0	0	Resistant
(500 ppm)	*S. aureus*	0	0	0	Resistant
	E. coli	0	0	0	Resistant
(1000 ppm)	*S. aureus*	0	0	0	Resistant
	E. coli	0	0	0	Resistant
Amoxicillin: 25 g (Positive control)	*S. aureus*	31.9			Susceptible
	E. coli	17			Susceptible

Table 2 illustrates that across all concentration samples of *C. crepidioides*, the negative control (DMSO) failed to produce any zones of inhibition in both gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria, resulting in a zero diameter. Conversely, the positive control (Amoxicillin) exhibited diameters of 17.0 mm and 31.9 mm against *E. coli* and *S. aureus*, respectively. The study utilized the Kirby-Bauer Disk Diffusion Susceptibility Test Protocol to interpret the data based on zone sizes, categorizing the results as Susceptible, Intermediate, or Resistant [26] [39]. Both bacterial strains resisted *C. crepidioides* leaves liquid extract concentrations (200 ppm, 500 ppm, and 1000 ppm) and the negative control (DMSO), indicating their lack of response to the treatments. Remarkably, the positive control, falling within the susceptibility range (≥13 - ≥29 mm), demonstrated susceptibility with a diameter of 17.0 mm on *E. coli* and 31.9 mm on *S. aureus.* The positive control displayed a larger diameter against *S. aureus* compared to *E. coli*, suggesting that gram-positive bacteria were more susceptible to elimination than gram-negative bacteria due to their distinct structure, which contributes significantly to global morbidity and mortality [40]. This outcome potentially stems from

various factors, including sample preparation, influencing the antibacterial activity of the liquid extract against the bacteria, corroborating the assertion that the zone size may be influenced by the size and weight of antibiotic molecules [41].

Cytotoxicity Analysis

Cytotoxicity studies looked into the potential toxicity of natural plant products on human cells. These studies were significant in determining the concentration at which natural plant products can be used safely without harming human cells [32]. The following tables summarized the mortality rate of brine shrimp nauplii treated with different concentrations of *C. crepidioides* leaves liquid extract, which provided insights into the extract's potential cytotoxicity.

Table 3. Mortality Rate of Brine Shrimp Nauplii After Treatment with the 6 Concentrations of *C. crepidioides* Leaves Liquid Extract

Concentrations (ppm)		R1	R2	R3	R4	R5	Overall mean
0	Mean (Alive)	7.7	7.7	6	6.3	6.7	6.9
	Mean (Death)	2.3	2.3	4	3.7	3.3	3.1
	Mortality Rate	23 %	23 %	40 %	37 %	33 %	32.2%
75	Mean (Death)	7.7	6	5.3	5	6	6
	Mean (Death)	2.3	4	4.7	5	4	4
	Mortality Rate	23 %	40 %	47 %	50 %	40 %	40%
200	Mean (Alive)	6.3	7	5	6	5.7	6
	Mean (Death)	3.7	3	5	4	4.3	4
	Mortality Rate	37 %	30 %	50 %	40 %	43 %	40%
500	Mean (Alive)	6	5	6.6	6	5.3	5.8
	Mean (Death)	4	5	3.4	4	4.7	4.2
	Mortality Rate	40 %	50 %	34 %	40 %	47 %	35%
750	Mean (Alive)	5	7	5.7	6.7	6.7	6.2
	Mean (Death)	5	3	4.3	3.3	3.3	3.8
	Mortality Rate	50 %	30 %	43 %	33 %	33 %	37.8%
1000	Mean (Alive)	5.7	5.7	4.3	5.3	6	5.4
	Mean (Death)	4.3	4.3	5.7	4.7	4	4.6
	Mortality Rate	43 %	43 %	57 %	47 %	40 %	46%
	LC_{50}	79432.82 ppm					
	Interpretation	Non-toxic					

Table 3 shows the cytotoxicity analysis of *C. crepidioides* leaves liquid extract, which revealed mean mortality rates ranging from 32.2 to 46 % across different concentrations. However, the LC_{50} 79432.82 ppm was considered non-toxic based on Meyer and Clarkson's level of toxicity, which suggests that the extract is safe for use [42]. This may be due to some bioactive compounds in the plant and other factors such as preparation, extraction, and analysis methods. Similarly, in *C. crepidioides* in vitro, the proliferation of S-180 cells is not inhibited. It was nontoxic to MRC-5 and HepG2 cell lines at a dose of 100 g/mL [43]. In contrast to other species, studies found cytotoxic effects in various plant extracts at different concentrations [44]. Interestingly, identified cytotoxicity in the petroleum ether extract of *H. littoralis* leaves showed LC_{50} values 273.77, 97.27, 51.60, 37.12, 14.60 and 12.59 ppm after 12, 18, 24, 30, 36 and 42 h [45]. Also, the study found cytotoxicity in the root, stem, and leaf extracts of *C. nardus*, with LC_{50} values ranging from 10.489 to 67.841 µg/ml. These studies collectively demonstrated the potential cytotoxicity of various plant extracts [46]. Nonetheless, studies have found cytotoxic effects in *L. cupanioides* and bitter leaf extracts at similar concentrations [47].

Furthermore, there is a 70 % cytotoxic effect at a 2 % concentration of *V. amygdalina* extract while *A. precatorius* leaf and root extracts showed significant toxicity with brine shrimp nauplii. The root and leaf n-hexane and dichloromethane fractions had LC_{50} values of 7.870 ppm and 19.135 ppm (ug/ml), respectively, indicating their exceptional potency [48]. However, the methanol fractions in the two extracts were less potent; for the root and leaves, the LC_{50} values were 41.575 ppm and 226.053 ppm (ug/ml), respectively [49]. The study found that *P. amarus* extract has the most cytotoxic activity among the plant extracts studied [50]. Also, the cytotoxic effect on human cancer cell lines was treated with *R. brachycarpum. D*. [51]. Another study found that 14 out of 16 Nigerian medicinal plant extracts were cytotoxic, with *Q. africana* and *Q. amara* stem bark extracts showing the most significant activity [52].

4. CONCLUSION AND RECOMMENDATIONS

Conclusions

Based on thorough analysis and further evaluation of the results and findings of the study, the following conclusions are drawn:

According to the data, alkaloids, tannins, and terpenoids were discovered through phytochemical screening of *C. crepidioides* leaves extract in each of five trials using Wagner, Ferric chloride, and Salkowski tests, respectively. The results showed that alkaloids, tannins, and terpenoids were indicated, leading to the conclusion that Redflower Ragleaf (*C. crepidioides*) was considered to have a therapeutic effect. The secondary metabolite components make it a potential antioxidant and potent nutritious food supplement that can be used as a new treatment and medicine.

The Kirby-Bauer technique was used to test the antibacterial activity of Redflower Ragleaf (*C. crepidioides*) leaves liquid extract against *E. coli* and *S. aureus*. Four different concentrations (0 ppm, 200 ppm, 500 ppm, and 1000 ppm) were tested, along with a positive control (Amoxicillin), and each concentration was tested three times. The results showed that the *C. crepidioides* liquid extract did not have any detectable antibacterial activity against the two tested bacterial strains, as there was no zone of inhibition throughout the test samples. The positive control (Amoxicillin) was effective in preventing the spread of bacterial strains, inhibiting a mean diameter of 17.0 mm on *E. coli* and 31.9 mm on *S. aureus*. The negative control allowed *E. coli* and *S. aureus* to grow, while the positive control

prevented their growth. These results suggest that Redflower Ragleaf lacks the necessary secondary metabolites for antibacterial properties and cannot be used as a pesticide.

Lastly, the Brine Shrimp Lethality Assay (BSLA) was conducted to evaluate the cytotoxicity of *C. crepidioides* at different concentrations. Six predetermined concentrations were used (0 ppm, 75 ppm, 200 ppm, 500 ppm, 750 ppm, and 1000 ppm) with five replicates for each concentration and three trials per replication. The test results showed that the brine shrimp's mean mortality rate ranged from 32.2 % to 46 %, and the LC_{50} was calculated to be 79,432.82 ppm. Based on the toxicity standard value established by Meyer and Clarkson's criteria, the extract was found to be non-toxic. This indicates that *C. crepidioides* would not be harmful to humans or animals if ingested or touched. Also, it is inferred that the active constituent and concentration of the plant contribute to its non-lethal nature.

Recommendations

To further explore Redflower Ragleaf's potential benefits, it is recommended to study its other parts, such as the flower, stem, and root. This can be done by investigating its secondary metabolites, such as flavonoids, saponins, and glycosides, and by combining its leaves' liquid extract with a plant that has antibacterial properties, like *Aloe vera*. Also, the study suggests using crude and solid extracts to analyze different activities such as hypertension, antidiabetic, antioxidants, and anti-inflammatory. Furthermore, to achieve different findings based on the measurement, it is advised to use other solvents such as methanol, water, hexane, petroleum ether, diethyl ether, n-heptane, isopropanol, acetone, and Tween 20 to modify the concentration.

For a thorough and precise phytochemical analysis, it is recommended to use different tests such as Ragendorff's reagent, Molisch's test, Liebermann-Burchard test, Shinoda test, and the Bornträger test.

In addition, to determine the antibacterial capability of Redflower Ragleaf, it is suggested to use different gram-negative bacteria, which include *P. aeruginosa*, *S. enterica*, *K. pneumoniae*, *E. cloacae*, *S. marcescens*, and *A. baumannii* alongside other gram-positive bacteria, such as *B. subtilus*, *E. faecalis*, *E. faecium*, *Corynebacterium spp*., and *S. pneumoniae*. Additionally, it is recommended to use different methods for assessing plants' antibacterial activity instead of relying solely on the Kirby-Bauer method.

To further explore the plant's toxicity, it is suggested that different test subjects such as mice, yellow fever mosquito larvae, and fish be used to measure the period of death depending on its varying concentrations. Other assays such as MTT, LDH, SRB, Alamar Blue, Clonogenic, and Comet assays can be used to test for the plant's toxicity.

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