A Study On Phytochemical Screening And Evaluation Of Pharmacological Activities Of Couroupita Guianensis Aubl. (Cannon Ball Tree)

Anjali B[1], Shalini C[2], Ranjitha CD[2], Keshamma E[3*]

[1]*Assistant Professor, Department of Biochemistry, Maharani's Science College for Women (Maharani Cluster University), Palace Road, Bengaluru, Karnataka, India.*
[2]*Postgraduate Student, Department of Biochemistry, Maharani's Science College for Women, Palace Road, Bengaluru, Karnataka, India.*
[3]*Associate Professor, Department of Biochemistry, Maharani's Science College for Women (Maharani Cluster University), Palace Road, Bengaluru, Karnataka, India.*
**Corresponding Author*
Dr. Keshamma E
Associate Professor, Department of Biochemistry,
Maharani's Science College for Women (Maharani Cluster University),
Palace Road, Bengaluru-560 001, Karnataka, India.
Email: keshamma.blr76@gmail.com

Keywords:
Couroupita guianensis, Fruit pulp, Bud, Antibacterial, Antioxidant, Antidiabetic

ABSTRACT
Several herbs have been used historically in Ayurvedic medicine to treat a wide range of illnesses. Moreover, multi-purpose medicinal plants have continued to elicit research interest and commercial attention globally as natural resources considered rich in nutritional and pharmacological properties to treat and cure several diseases. Therefore, we aimed to screen for the presence of phytochemicals in Couroupita guianensis and to evaluate its pharmacological activities. Different parts of C. guianensis were subjected to solvent extraction by maceration with various solvents such as water, ethanol, methanol and acetone. The pharmacological activities viz. antibacterial, antioxidant, and antidiabetic activities of selected C. guianensis extracts were evaluated invitro. The methanolic fruit pulp extract of C. guianensis yielded maximum extract (27.1%) followed by aqueous (aq.) fruit pulp extract of C. guianensis (26%), and ethanolic fruit pulp extract of C. guianensis (19.4%). The aq. bud extract of C. guianensis was selected for further evaluation of pharmacological activities because it contains a greater number of phytochemicals such as alkaloids, glycosides, phenolic compounds and tannins. Similarly, the methanolic fruit pulp extract of C. guianensis was selected for further pharmacological activities evaluation because it contains a greater number of phytochemicals such as alkaloids, phytosterols, phenolic compounds, anthraquinone, terpenoids and tannins. Both the extracts exhibited inhibitory activity against all the test bacterium viz. S. aureus, Bacillus sp., Pseudomonas sp. and E. coli but with a varied zone of inhibition. The aq. bud extract of C. guianensis showed higher FRAP (1.17 ± 0.02), when compared to methanolic fruit pulp extract of C. guianensis (0.49 ± 0.02). The aq. bud extract of C. guianensis showed highest alpha-amylase inhibition percentage (72.88 ± 0.0) at 9mg/0.5ml as compared to methanolic fruit pulp extract of C. guianensis (56.55 ± 1.16). In conclusion, C. guianensis appears to be a promising resource for bioactive agents, which can be exploited for the prevention and treatment of oxidative stress and dreadful diseases.

INTRODUCTION

Multi-purpose medicinal plants have continued to elicit research interest and commercial attention globally as natural resources considered rich in nutritional and pharmacological properties to treat and cure several diseases.[1] Plants can synthesize different bioactive molecules such as phenols, flavonoids, vitamins, alkaloids, terpenoids, tannins, glycosides, quinones and many others. Most of the plants used for medicinal purposes have been identified and their uses are well documented.[2] Furthermore, most drugs in the past i.e., allopathic, ayurvedic and homeopathic medicines were made from plants.[3]

High intake of natural products is associated with reduced risk of a number of chronic diseases such as artherosclerosis and cancer. Recently, consumers have been more concerned about the addition of synthetic additives to food and the two most commonly used antioxidants, butylated hydroxyanisole and butylated hydroxytoluene have shown DNA damage induction. Therefore, an interest is growing for the search of natural antioxidants for the public perception that natural and dietary antioxidants are safer than synthetic analogues.[4]

Since the discovery of traditional antibiotics (such as penicillin), many microorganisms are now resistant to one or more antibacterial drugs. Antimicrobial resistance proves to cause fatal injuries to thousands of people every year, leading to high medical costs and serious economic losses. The increasing failure of chemotherapeutic drugs and the antibiotic resistance of pathogenic microorganisms has led to some medicinal plants being studied for their potential antibacterial activities.[3,5] The utilization of plant extracts and phytochemicals, both with known antimicrobial properties, can be of incredible importance in therapeutic medicines.[6]

Diabetes mellitus is a chronic metabolic disorder and is characterized by high blood glucose level which results from defects in both insulin secretion and/or insulin action.[7] Type 2 Diabetes affects 90-95% of people, and it is a most critical disorder throughout the world with India listed in the top three countries.[8,9] Several pathological processes are involved in the development of diabetes. Various long-term complications of diabetes developed due to the chronic hyperglycemia and insulin resistance.[8] The core remedy for managing diabetes is to lower hyperglycaemia and reduce intestinal glucose absorption through the inhibition of carbohydrate metabolizing enzymes viz. alpha-amylase and alpha-glucosidase.[10]

Herbal medicine is still the mainstay of treatment in about 75%–80% of people in many developing countries for their primary health care because of better cultural acceptability and compatibility with the human body and fewer side effects. There are different ways in which plants have been found useful in medicines. The parts of medicinal plants that may be used are different types of seeds, root, leaf, fruit, flowers or even the whole plant.[2] Couroupita guianensis is cultivated as an ornamental tree for its beautiful flowers in the Indian Gardens. In the pharmaceutical industry these plants are known because of their broad spectrum of structural diversity and their wide variety of pharmacological activities (Figure 1).[11,12]

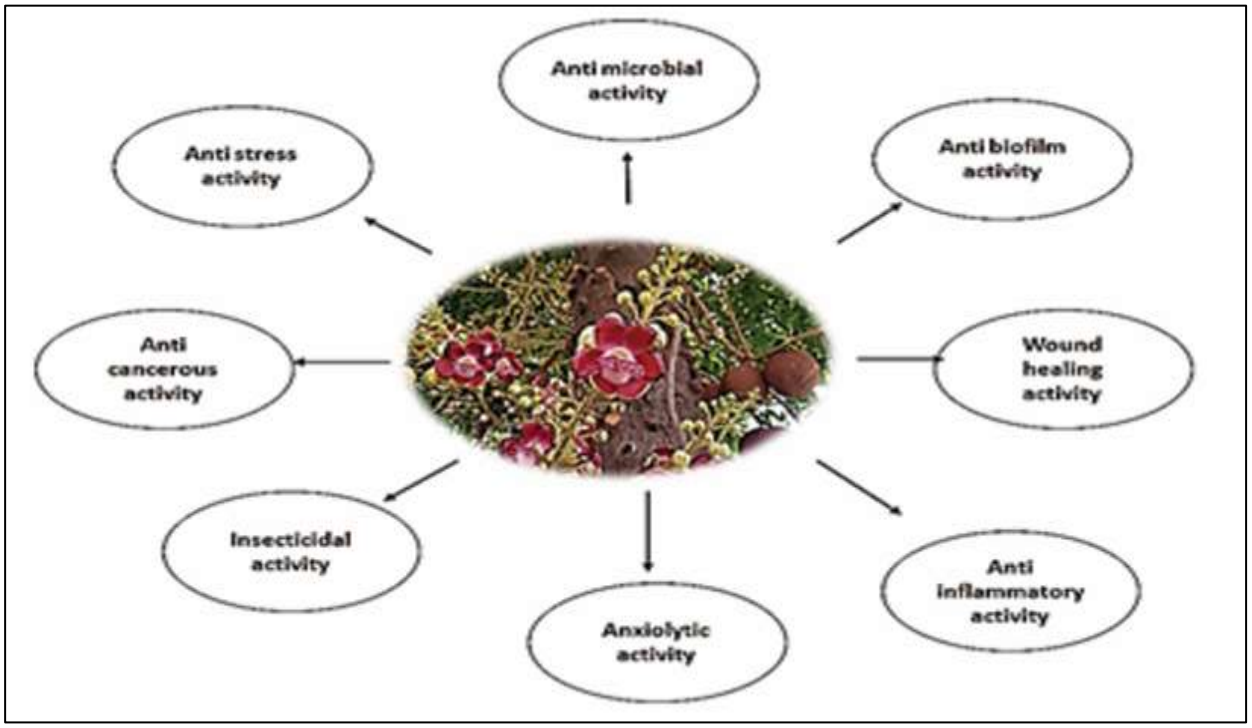

Figure 1. Illustration of pharmacological activities of C. guianensis[11,12]

With this scenario, present study was conducted with the main objectives to screen for the presence of phytochemicals in C. guianensis and to evaluate its pharmacological activities.

MATERIALS AND METHODS

Collection and Identification of Plant

For identification, flowering twig, leaves, bark, inflorescence and fruits of the tree (Figure 2) were collected from Maharani's Science College for Women campus, Bengaluru-01 and submitted to Dr. Suresh Kumar, Professor, Department of Botany, Maharani's Science College for Women, Maharani Cluster University, Bengaluru, Karnataka. The plant was authenticated as Couroupita guianensis Abul. (Cannon ball tree).

Figure 2. Showing C. guianensis whole tree and its parts

A. Whole tree, B. Fruits, C. Inflorescence, D. Leaves, and E. Flower

Extraction

Different parts of C. guianensis were collected, washed and shade dried for a week. The dried parts were powdered using a blender. The powdered samples were stored in airtight containers until further use. 20g of powdered sample was taken in a conical flask and 200ml of different extraction solvent (water, methanol, ethanol, and acetone) was added and the conical flask was placed in a orbital shaker for 48 hours at 37°C. There after the extract was filtered using Whatman No.1 filter paper and allowed to air dry in petri plates. The yield (%) was calculated by using the following formula:

Yield (%) = (weight of extract/weight of sample) × 100

Phytochemical Screening

The phytochemical screening of extracts of C. guianensis was carried out using standard procedures to detect phytoconstituents as described by Sofowora,[13] Trease and Evans[14] and Harborne.[15]

Assay of Antibacterial Activity

The sensitivity of different bacterial strains to extracts of C. guianensis was measured in terms of zone of inhibition using agar diffusion assay as described by Kavitha et al.[16] Briefly, plates containing Mueller-Hinton agar were spread with 50µl of the inoculum. Wells (8mm diameter) were cut out from agar plates using a sterilized stainless steel cork borer and filled with the extracts and other components (DMSO, Ofloxacin, and extracts of C. guianensis). The plates were inoculated with different bacteria, and then incubated at 37°C for 24 hours. The diameter of any resultant zone of inhibition was measured. For each combination of extract and the bacterial strain, the experiment was performed in duplicates. The bacteria with a clear zone of inhibition of more than 12mm was considered to be sensitive. The antibacterial activity of different solvent extracts was compared with standard antibiotic (Ofloxacin).

Assay of Antioxidant Activity

The Ferric reducing antioxidant power (FRAP) assay of extracts of C. guianensis was carried as per standardized method described by Rokkam et al.[17] Briefly, 1ml of different concentration of ascorbic acid (20µg, 40µg, 60µg, 80µg, and 100µg) was taken in a 10ml volumetric flask and mixed with 2.5ml of 0.2M phosphate buffer (pH 6.6). To this 2.5ml of 1% potassium ferricyanide solution was added. The mixture was incubated for 20 minutes at 50°C. After incubation, 2.5ml of 10% trichloro acetic acid (TCA) was added to stop the reaction. The above mixture was centrifuged at 1650rpm for 10 min. From the upper layer solution 2.5ml was taken and mixed with 2.5ml of 0.1% Ferric chloride solution and absorbance was recorded at 700nm using a suitable blank. Different concentrations of aq. bud and methanolic fruit pulp extracts of C. guianensis (20µg, 40µg, 60µg, 80µg, and 100µg) were taken and processed similar to the standards as described above. The FRAP value was calculated using the following formula:

FRAP value = (Abs_{sample} / $Abs_{standard}$) × FRAP value of standard*

*FRAP value of ascorbic acid is 2

Assay of Alpha-amylase Inhibition Activity

The alpha-amylase inhibition activity of extracts of C. guianensis was assayed as per standardized method described by Suthindhiran et al.[18] Briefly, 500µl of extracts of C. guianensis of different concentration (5mg, 7mg, and 9mg) and 500µl of 0.02M Sodium phosphate buffer (pH 6.9) containing alpha-amylase (0.5 mg/ml) were incubated at 25°C for 10 minutes. After pre-incubation 500µl of 1% starch solution in 0.02M phosphate buffer was added to the reaction, and the reaction was stopped by adding 1ml of DNS reagent. The test tubes were then incubated in a boiling water bath for 5 min and

cooled to room temperature. The reaction mixture was then diluted by adding 10ml distilled water and absorbance was measured at 540nm. The inhibition (%) was calculated using the following formula:

% Inhibition = [$Abs_{control}$ –$Abs_{extract}$ /$Abs_{control}$] × 100

RESULTS

The yield of different parts of C. guianensis in different solvents is represented in Table 1. Results depicted that methanolic fruit pulp extract of C. guianensis yielded maximum extract (27.1%) followed by aq. fruit pulp extract of C. guianensis (26%), and ethanolic fruit pulp extract of C. guianensis (19.4%).

Table 1: Yield of different parts of C. guianensis in different extraction solvents

Extracts	Colour	Yield (%)
Methanol extract of fruit pulp	Reddish brown	27.10
Aq. extract of fruit pulp	Ash or Grey	26.00
Ethanol extract of fruit pulp	Reddish brown	19.40
Acetone extract of fruit pulp	Reddish brown	14.50
Aq. extract of whole flower	Ash or grey	12.30
Aq. extract of bud	Black	11.40
Aq. extract of leaves	Green	10.50
Methanol extract of bud	Light brown	5.00
Ethanol extract of bud	Light brown	4.90
Acetone extract of bud	Light brown	2.10
Aq. extract of bark	Brown	1.20

Aq. Aqueous

The aqueous, ethanol, methanol and acetone extracts of bud of C. guianensis were investigated for phytochemicals. The aq. extract contains alkaloids, carbohydrates, glycosides, proteins, phenolic compounds and tannins. Ethanol extract contains carbohydrates, proteins, phenolic compounds, anthraquinone and tannins. Methanol extract contains alkaloids, carbohydrates, proteins, phenolic compounds, anthraquinone and tannins. Acetone extract contains alkaloids, carbohydrates, proteins, phenolic compounds and tannins. The aq. bud extract of C. guianensis was selected for further evaluation of pharmacological activities because it contains a greater number of phytochemicals (Table 2).

Table 2: Phytochemical screening of bud extract of C. guianensis

Tests	Extraction Solvent			
	Aqueous	Ethanol	Methanol	Acetone
Alkaloids	+	-	+	+
Carbohydrates	+	+	+	+

Glycosides	+	-	-	-
Saponins	-	-	-	-
Proteins & Amino acids	+	+	+	+
Phytosterols	-	-	-	-
Fixed oils & Fats	-	-	-	-
Phenolic compounds	+	+	+	+
Flavonoids	-	-	-	-
Anthraquinone	-	+	+	-
Terpenoids	-	-	-	-
Tannins	+	+	+	+

(+): Positive (Present); (-): Negative (Absent)

The aqueous, ethanol, methanol and acetone extracts of fruit pulp of C. guianensis were investigated for phytochemicals. The aq. extract contains carbohydrates, proteins, phytosterols, phenolic compounds and tannins. Ethanol extract contains alkaloids, carbohydrates, proteins, phenolic compounds, phytosterols, oils, terpenoids and tannins. Methanol extract contains alkaloids, carbohydrates, proteins, phytosterols, oils, phenolic compounds, anthraquinone, terpenoids and tannins. Acetone extract contains carbohydrates, phytosterols, phenolic compounds, anthraquinone and tannins. The methanolic fruit pulp extract of C. guianensis was selected for further pharmacological activities evaluation because it contains a greater number of phytochemicals (Table 3).

Table 3: Phytochemical screening of fruit pulp extract of C. guianensis

Tests	**Extraction Solvents**			
	Aqueous	**Ethanol**	**Methanol**	**Acetone**
Alkaloids	-	+	+	-
Carbohydrates	+	+	+	+
Glycosides	-	-	-	-
Saponins	-	-	-	-
Proteins & Amino acids	+	+	+	-
Phytosterols	+	+	+	+
Fixed oils & Fats	-	+	+	+
Phenolic compounds	+	+	+	+
Flavonoids	-	-	-	-
Anthraquinone	-	-	+	+

Terpenoids	-	+	+	-
Tannins	+	+	+	+

(+): Positive (Present); (-): Negative (Absent)

The result of inhibitory activity of aq. bud extract and methanolic fruit pulp extract of C. guianensis are shown in Table 4. Results revealed that both the extracts exhibited inhibitory activity against all the test bacterium viz. S. aureus, Bacillus sp., Pseudomonas sp., and E. coli but with a varied zone of inhibition. Overall, both the extracts displayed marked inhibitory activity against the test bacterium in comparison with reference (Ofloxacin). The inhibitory activity of reference (Ofloxacin) was higher when compared to plant extracts. S. aureus showed higher sensitivity to methanolic fruit pulp extract of C. guianensis compared to aq. bud extract of C. guianensis. Bacillus sp. showed higher sensitivity to aq. bud extract of C. guianensis when compared to methanolic fruit pulp extract of C. guianensis. Pseudomonas sp. and E. coli showed similar sensitivity for both the extracts.

Table 4. Antibacterial activity of C. guianensis extracts

	Zone of Inhibition (mm)			
Test Organisms	**Aq. bud extract**	**Methanolic fruit extract**	**Antibiotic (Ofloxacin)**	**DMSO**
S. aureus	17.5 ± 0.70	19.5 ± 0.71	32.0 ± 0.00	0.0
Bacillus sp.	15.5 ± 0.70	14.5 ± 0.71	30.0 ± 0.00	0.0
Pseudomonas sp.	14.5 ± 0.70	15.0 ± 0.70	34.0 ± 1.41	0.0
E. coli	16.5 ± 0.70	16.0 ± 1.41	35.0 ± 0.00	0.0

Values were expressed as mean; n=2; DMSO, Dimethyl sulfoxide

The results of FRAP assay of aq. bud extract of C. guianensis and methanolic fruit pulp extract of C. guianensis is depicted in Figure 3. The extracts exhibited a dose dependent FRAP with increase in the concentration of extracts. The aq. bud extract of C. guianensis showed higher FRAP (1.17 ± 0.02), when compared to methanolic fruit pulp extract of C. guianensis (0.49 ± 0.02).

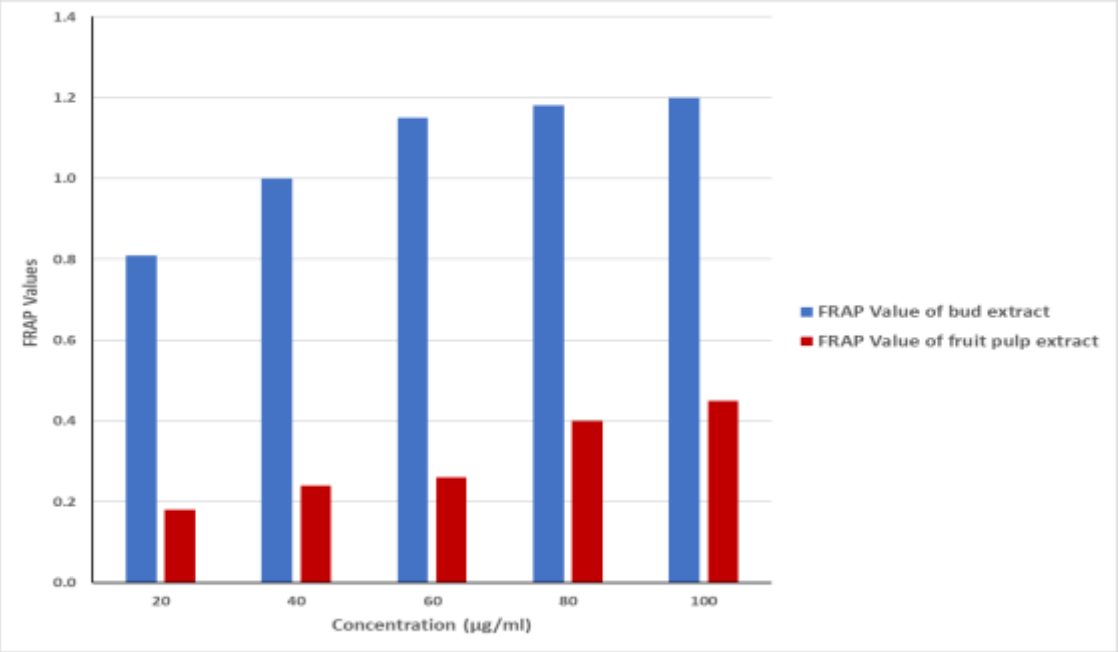

Figure 3. FRAP antioxidant assay of C. guianensis extracts

The methanolic fruit pulp extract of C. guianensis and aq. bud extract of C. guianensis caused dose dependent inhibition of alpha-amylase. Furthermore, the aq. bud extract of C. guianensis showed highest alpha-amylase inhibition percentage (72.88 ± 0.0) at 9mg/0.5ml as compared to methanolic fruit pulp extract of C. guianensis (56.55±1.16) in comparison with the standard drug Acarbose at 0.10mg/ml and 0.50mg/ml respectively (Figure 4).

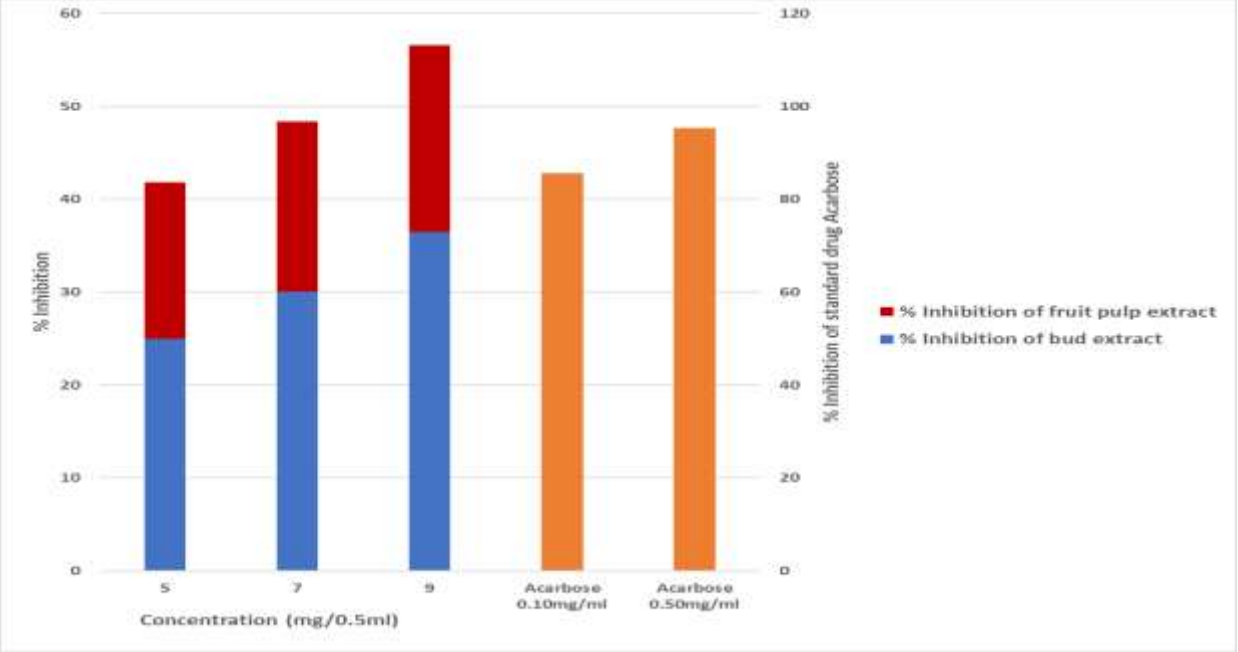

Figure 4. Alpha-amylase inhibition assay of C. guianensis extracts

DISCUSSION

Several herbs have been used historically in Ayurvedic medicine to treat a wide range of illnesses. The greatest bioresource for pharmaceutical intermediates, modern and traditional medicine, nutraceuticals,

food supplements, folk remedies, and chemical entities for synthetic drugs is found in medicinal plants.[9] Literature reports evidenced that C. guianensis is seen to have some pharmacological activities like antimicrobial, antifungal, antioxidant, antidiabetic, anti-inflammatory and anticancer.[19] Hence in the current study we aimed to screen for the presence of phytochemicals in C. guianensis extracts and to evaluate their pharmacological activities.

The medical pharmacological properties exhibited by plants are due to the presence of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, terpenoids etc…, that are distributed in the various parts of the plants that serve as defense mechanism against predation by many microorganisms. These chemicals are studied under the concept called phytochemicals which were detected in extracts of C. guianensis. Phenolic compounds, tannins, proteins, carbohydrates are present in different parts of this medicinally important plant. A prominent presence of phenolic compounds was observed in acetone and methanolic extracts of fruit pulp and also in aqueous and methanolic extracts of the bud. Terpenoids and phytosterols were detected in water, methanol, ethanol and acetone extract of the fruit pulp. Similar results were observed in the phytochemical screening results reported by Alagesaboopathi et al., (2013) wherein authors found presence of medicinally active constituents like alkaloids, flavonoids, tannins, phlobatannins, steroids, terpenoids and absence of saponins in the aqueous extract of leaf and fruit pulp extracts of C. guianensis.[20]

Until the mid 20th century bacterial diseases were probably the leading cause of death. Improved sanitation, vaccines and antibiotics have all decreased the mortality rates from bacterial infections. The emergence of multidrug resistant pathogens adversely affects the efficiency of many antibiotics. Herbal medicines are used for the treatment of many infectious diseases throughout the history of mankind. The increasing antibiotic resistance exhibited by microorganisms has led to the phytochemical screening of medicinal plants for antimicrobial activity. Many infections can be treated by phytochemicals possessing potent antimicrobial efficacy.[21] Furthermore, Akila and Keshamma (2019) opinioned that it is crucial to conduct research to examine the biological effects of medicinal plants against different pathogenic organisms and to discover novel antimicrobial compounds.[22]

In our study, antibacterial activity of bud and fruit pulp extracts of C. guianensis against gram-positive and gram-negative bacteria by agar well diffusion assay was determined. All the test organisms showed sensitivity to both the extracts. Highest antibacterial activity was observed in aq. bud extract of C. guianensis and methanolic fruit pulp extract of C. guianensis for S. aureus. The preliminary biochemical tests of methanolic extract of C. guianensis flowers showed the presence of glycosides, tannins, and phenolics, which group of compounds have previously been reported to exhibit anti-microbial effects.[23]

FRAP assay is a technique used to measure the antioxidant reducing power of a particular compound. The Reducing power is based on the substances which have reduction potential, that reacts with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanide (Fe^{2+}), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. In the present study, using this method ferric reducing antioxidant power of C. guianensis extracts was determined.[24]

Sumathi and Anuradha reported the antioxidant activity of methanolic flower extract of C. guianensis in different in-vitro studies. The methanolic flower extract of C. guianensis were influenced a strong antioxidant activity in the method of DPPH radical scavenging action when paralleled with ascorbic acid.[25] In another study carried out by Stalin et al., the antioxidant efficacy of ethanolic leaf and flower extract of C. guianensis in-vitro antioxidant activity such as reducing power ability, NBT reduction assay, deoxyribose degradation assay was performed and concluded that the ethanolic extract of leaves and flowers of C. guianensis showed significant antioxidant activity.[26] Similarly in our present study it was observed that aq. bud extract of C. guianensis and methanolic fruit pulp extract of C. guianensis exhibited antioxidant activity as determined by the FRAP assay.

One of the therapeutic approaches for decreasing of blood glucose rise after a meal is to retard the absorption of glucose by inhibition of carbohydrate hydrolyzing enzymes such as alpha-amylase and

alpha-glucosidase. The use of synthetic drugs viz. acarbose, miglitol and voglibose are the regular practices in control of postprandial hyperglycemia despite its gastrointestinal adverse effects. Hence, the research for new group of agents from natural resources especially from traditional medicines became an alternative approach for the treatment of postprandial hyperglycemia.[7]

Thus, the results from our study showed that the aq. bud extract of C. guianensis possesses significant in-vitro antidiabetic activity compared to methanolic fruit pulp extract of C. guianensis. Similar reports suggests that the plant extracts can act as a potent antidiabetic agent.[27] C. guianensis flowers that have been shown to promote wound healing, reduce inflammation, encourage the production of keratinocytes, and stimulate the proliferation of fibroblasts.[28]

CONCLUSION

In conclusion, our study findings revealed presence of major secondary metabolites in medicinal plant C. guianensis and demonstrated antibacterial, antioxidant, and antidiabetic potential of C. guianensis. Hence, this medicinal plant appears to be a promising resource for bioactive agents, which can be exploited for the prevention and treatment of oxidative stress and dreadful diseases. However, further studies on isolation of bioactives from C. guianensis, characterization, and evaluation of pharmacological activities in-vivo are warranted.

REFERENCES

1. Keshamma E, Shobha Rani K. Phytochemical Evaluation and Determination of Antioxidant Activities of Moringa Oleifera. International Journal of Food and Nutritional Sciences. 2021;10(12):866-872.
2. Keshamma E. Evaluation of Antimicrobial and Antioxidant Properties of Stem Extracts of Tinospora Cordifolia (Amrutha Balli)). International Journal of Food and Nutritional Sciences. 2022;11(10):1922-1930.
3. Keshamma E, Suresh Kumar C, Manjula AC, Prathibha KY. Phytochemical Screening and Determination of Antibacterial Activities of Silver Nanoparticles of White Button Mushroom (Agaricus bisporus). International Journal of Innovative Research in Technology. 2021; 8(4):490-495.
4. Akhila A, Keshamma E. A Study on Phytochemical Analysis and Determination of Antioxidant Antimicrobial Activities of Coriandrum sativum (Dhania). International Journal of Current Microbiology and Applied Sciences. 2019;8(10):2755-2763.
5. Iwalokun BA, Usen UA, Otunba AA, Olukoya DK. Comparative phytochemical evaluation, antimicrobial and antioxidant properties of Pleurotus ostreatus. African journal of Biotechnology. 2007;6(15).
6. Vanitha NM, Keshamma E. Assay of Antimicrobial Activity of Leaf Extract of Madhunasini (Gymnema sylvestre). International Journal of Current Microbiology and Applied Sciences. 2015; 4(5):1222-1226.
7. Veena MR, Keshamma E. Phytochemical Analysis and Evaluation of In-vitro Alpha-amylase Inhibition Activity of Rhizome Extract of Curcuma longa (Turmeric). International Journal of Current Microbiology and Applied Sciences. 2019;8(08):3130-3137.
8. Keshamma E, Kamal Kant Patra. A Study on Evaluation of Antidiabetic Potential of Cuminum cyminum (Cumin). International Journal of Current Microbiology and Applied Sciences. 2016;5(10):1136-1142.
9. Sumangala N, Keshamma E. A Study on Evaluation of In-Vitro Antidiabetic Activities of Endangered Plant Species Syzygium Travancoricum of the Western Ghats. Journal of Chemical Health Risks. 2024;14(3): 3483-3487.
10. Sajeeda Niketh, Rajeev Ramachandra Kolgi, Shobha N, Keshamma E. Assessment of Antidiabetic Potential of Aqueous Leaf Extract of Moringa Oleifera by In Vitro Experimental Methods. International Journal of Food and Nutritional Sciences. 2022;11(12):18011-18022.
11. Jagannatha S, Maity S, Santra A, Kuruvalli G, Reddy VD, Golla R. An Updated Review on Ethnomedicinal Uses Phytochemistry and Pharmacological Activities of Couroupita guianensis. Pharmacognosy Reviews. 2024;18(36):111-6.
12. Krishnananda Ingle P, Amit Deshmukh G, Dipika Padole A, Mahendra Dudhare S, Mangesh P, Mohariland Vaibhav, Khelurkar C. Phytochemicals: Extraction Methods, Identification and Detection of Bioactive Compounds from Plant Extracts. Journal of Pharmacognosy and Phytochemistry. 2016;6(1):32-36.
13. Sofowora A. Medicinal plants and Traditional Medicine in Afric. John Wiley Son Ltd. 1993:150-3.

14. Trease GE, Evans WC. Pharmaccology, 11th Edtn. London: Brailliar Tiridel and Macmillian Publishers; 1989.
15. Herborne JB. Phytochemical methods. 3rd ed D.E. and Hall Ltd. London; 1973. p. 135-203.
16. Kavitha R, Kamalakannan P, Deepa T, Elamathi R, Sridhar S, Suresh Kumar J. In vitro antimicrobial activity and phytochemical analysis of Indian medicinal plant Couroupita guianensis Aubl. Journal of Chemical and Pharmaceutical Research. 2011;3(6):115-21.
17. Rokkam R, Pinipay F, Bobbili S, Chokkandla R, Tamanam RR. Delineating Phytochemical, Antioxidant, And Antiinflammatory Properties of Couroupita Guianensis Flower Parts. International Journal of Creative Research Thoughts. 2023;11(5):251-258.
18. Suthindhiran KR, Jayasri MA, Kannabiran K. α-glucosidase and α-amylase inhibitory activity of Micromonospora sp. VITSDK3 (EU551238). International Journal of Integrative Biology. 2009;6(3):115-20.
19. Chauhan HH, Chavan MD, Choudhary RR, Madkaikar HM, Dalvi TS, Shah NJ. Couroupita Guianensis – It's Ethnobotanical Knowledge, Phytochemical Studies, Pharmacological Aspects and Future Prospects. Journal of Emerging Technologies and Innovative Research. 2022; 9(4):407-416.
20. Alagesaboopathi C. Phytochemical screening and antibacterial potential of Couroupita guianensis Aubl. and Erythroxylum monogynum Roxb. International Journal of Current Research. 2013; 5:2068-71.
21. Varghese G. Challenges in Managing Multidrug-Resistant Gram-Negative Infections. International Journal of Infectious Diseases. 2025; 152:107820.
22. Akhila A, Keshamma E. Evaluation of Antioxidant and Antibacterial Activities of Emblica officinalis (Amla). International Journal of Current Microbiology and Applied Sciences. 2019;8(09):3061-3068.
23. Majumder S, Elango EM, Hoebe CJ, Rahmatullah M. Antibacterial studies with methanol extract of Couroupita guianensis flowers against methicillin-resistant Staphylococcus aureus. World Journal of Pharmacy and Pharmaceutical Sciences. 2014;3(9):543-550.
24. Dontha S. A review on antioxidant methods. Asian Journal of Pharmaceutical and Clinical Research. 2016;9(2):14-32.
25. Sumathi S, Anuradha R. Invitro antioxidant activity of methanolic extract of flower of Couroupita guinensis Aubl. Journal of Chemical and Pharmaceutical Research. 2016;8(5):618-23.
26. Stalin C, Vishnuvardhan T, Sravyamounika K, Roby KA, Prasanna TL. Phytochemical Screening and antioxidant activity of flowers and leaves of Couroupita guianensis Aubl. International Journal of Phytopharmacy Research. 2012;3(1):20-3.
27. Somani G, Chaudhari RA, Sancheti JA, Sathaye SA. Inhibition of carbohydrate hydrolyzing enzymes by methanolic extract of Couroupita guianensis leaves. International Journal of Pharmacy and Biological Sciences. 2012;3(4):511-20.
28. Santwana Palai, Sk Abdul Rashid, Ritun Patra, Sarita Jena, Biswakanth Kar, Sabitri Bindhani, Kautuk Kumar Sardar. Biological activities and diabetic wound healing potential of Couroupita guianensis Aubl. flowers: Current knowledge and concepts. Annals of Phytomedicine. 2024;13(2):44-54.