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DELINEATING PHYTOCHEMICAL, ANTIOXIDANT, AND ANTIINFLAMMATORY PROPERTIES OF *COUROUPITA GUIANENSIS* FLOWER PARTS.

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Abstract: This study aimed to determine the total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC), and antioxidant and anti-inflammatory properties of *Couroupita guianensis*., and aims at the novel plant sources (parts of the flower) which are utilized for their therapeutic applications. The parts of the flower (male, female, and petals) of *C. guianensis* were extracted by methanolic maceration. Folin-Ciocalteu (FC) assay was used for determining the TPC and TTC whereas aluminum chloride colorimetric assay was used for determining TFC. Total Antioxidant (TA) assay, Ferric reducing antioxidant power (FRAP), and Reducing power (RP) assays were used for estimating antioxidant activity. Anti-inflammatory was evaluated by heat-induced hemolysis assay (HRBC) and Egg albumin denaturation assay. The result of three extracts from *C. guianensis* showed that the highest TPC, TFC, TTC, TA, FRAP and inhibition RBC hemolysis of the petals were 27.62 ± 5.516 mg GAE/g extract, 55.21 ± 10.05 mg TAE/g extract, 18.60 ± 7.23 mg QE/g extract, 2.98 ± 0.245 AAEq mg/ml extract, 374.20 ± 7.190 FRAP Units and 97.75 ± 0.09% (1000 µg/ml) with an IC_{50} (half maximal inhibitory concentration) of 329.96 ± 0.5234 µg/mL, respectively. The Female part showed that the strongest activity of reducing power and inhibition of protein denaturation was 0.37 ± 0.027 AAEq mg/ml extract and 29.55 ± 0.027%, with an IC_{50} of 1579.23 ± 1.253 µg/mL, respectively. The conclusion of this study indicates that the petals of *C. guianensis* flower extracts have high potential as novel pharmaceutical applications for antioxidant and anti-inflammatory properties.

***Index Terms - Couroupita guianensis*, Phytochemical, Antioxidants, Anti-inflammatory, Flower.**

I. INTRODUCTION

Since the beginning of time, different ailments have been treated with plants by several practices. The earliest practices that are still practiced today include Ayurveda, Traditional Indian Medicine (TIM), and Traditional Chinese Medicine (TCM), which date back to 4500 BC (Pandey et al., 2013). The quest for phytochemicals as an alternative to synthetic substances, which are frequently utilized in the food, pharmaceutical, and cosmetic industries, is gaining popularity today. The general public's opinions have changed because of the "green" movement in Western civilization, and many now believe that natural ingredients and extracts are fundamentally safer and superior to manufactured chemicals, with the net result being increased sales of herbal treatments (Atanasov et al., 2015). Medicinal plants are a valuable new source of lead compounds for potential therapeutic targets identified by genomics, proteomics, and high-throughput screening due to their structural diversity.

Hence, several herbal medicines have been proven to have anti-inflammatory and/or antioxidant benefits, even though the precise mechanism of action of these drugs is yet unknown (Choudhari et al., 2020). Despite limited awareness of their medical value, Flowers have been utilized for a variety of diseases since ancient times and they sometimes have characteristics that are different from those of other plant parts. Whether directly or indirectly, flowers have a significant impact on our daily life (Petrovska et al., 2012). The plant species *Couroupita guianensis* belongs to the Lecythidaceae family. Its native habitats are southern India and Malaysia, and it is frequently referred to in Telugu as Nagalinga pushpam. *C. guianensis* has enormous 3" to 5" waxy, fragrant flowers that develop right on the stem's bark (cauliflora). The flowers of *C. guianensis* have a red exterior with a tinge of yellow, are fragrant, and have stamens that are continued as the main androphore. They are rich in alkaloids, phenolics, flavonoids, and stigmasterol, and have essential active components namely isatin and indirubin. Several investigations have shown the presence of carbohydrates, proteins, α-amirin, β-amirin, β-sitosterol, ketosteroids, tannins, and terpenoids (Bergman, 2014).

The leaves and flowers of *C. guianensis* are used for therapeutic purposes, including the treatment of diseases, tumors, pain and inflammatory conditions, colds, intestinal gas production, and colic (Sanz et al., 2009; Prabhu et al., 2012). The volatile oils of the flower display antibacterial and antifungal effects and so they are used for the treatment of rashes, hemorrhages, scabies, diarrhea, and scorpion venom (Shah et al., 2012). As a result, the current study focuses on both qualitative and quantitative phytochemical investigation, the antioxidant and anti-inflammatory activity of methanol (MeOH) extracts isolated from the parts of flowers, namely Male (fertile stamens and staminodes), female (ovary), and petals of *C. guianensis*.

II. MATERIALS AND METHODS

2.1 PLANT MATERIALS

Mature flowers of *C. guianensis* were obtained on 2nd March 23, 2023, from the Andhra University, South Campus region (17° 43' 47.3880" N, 83° 19' 17.3820" E), Visakhapatnam, India. The plant was authenticated by the Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh, India based on its inflorescence. The collected fresh flowers were packed in a paper bag and stored in the laboratory at 2 to 8 °C of temperature and 90 to 95 % of relative humidity, until further use. Unspoiled flowers were selected, and they were dismembered into three distinct parts, namely male, female, and petals.

2.2 CHEMICALS AND INSTRUMENTS

Reagents and chemicals used for qualitative and quantitative estimation of phytochemicals, antioxidants, and antiinflammatory studies like Folin-Ciocalteu (FC) reagent, Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), Tannic acid, Gallic acid, Diclofenac sodium were purchased from Himedia Laboratories Pvt Ltd, Mumbai, India. The rest of the chemicals were of analytical grade and obtained from commercial sources. UV/Visible spectrophotometer (Shimadzu, UV-1800, Japan) was used for recording absorbance.

2.3 EXTRACTION

Separated flower parts (male, female, petals) of *C. guianensis* were air-dried for 3 to 4 days and then pulverized with a mechanical grinder. The crushed flower parts were macerated individually in 250 ml of most polar analytical grade methanol (0.762; polarity value) as a menstruum for 72 hours, with occasional stirring every 2 hours. After 72 hours the menstruum was double-filtrated using cheesecloth and filter paper. The collected menstruum of all three flower parts was distilled and separated extracts were stored at - 4 °C before use. Samples were prepared in Dimethyl sulfoxide (DMSO) for phytochemical analysis and antioxidant assays and whereas in distilled water for anti-inflammatory assays.

2.4 PHYTOCHEMICAL ANALYSIS

2.4.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS

Flower extracts of C. guianensis were analyzed qualitatively for the existence of phytochemicals; Tannins, Cardiac Glycosides, Phenols, Carbohydrates, Saponins, Flavonoids, Alkaloids, and Terpenoids. The presence of Tannins and Phenols was determined by ferric chloride ($FeCl_3$), Carbohydrates were determined by Benedict's test, Cardiac glycosides by the Keller-Killani test, Saponins is determined by their foaming ability, alkaloids were by the Dragendorff reagent, Flavonoids by the lead acetate test (Cho et al., 2003).

2.4.2 DETERMINATION OF TOTAL PHENOLIC CONTENT

Total phenolic content (TPC) was quantified by using the FC method (Javanmardi J et al., 2003). In this procedure, 0.2 µL of *C. guianensis* flower extracts were added to 1 mL of FC reagent and 0.8 µL of 7.5% Na_2CO_3. The optical density (OD) of reaction mixtures was measured at 765 nm. Gallic acid (20-200 µg/mL) was used as a standard, and the regression equation of the calibration curve is $y = 0.0094x + 0.1213$ with an R^2 of 0.9638. Results of TPC were reported in terms of Gallic acid equivalents (mg GAE/g) flower extract.

2.4.3 DETERMINATION OF TOTAL TANNIN CONTENT

Total tannin content (TTC) was quantified by using the FC method (Folin O, Ciocalteu V, 1927). In this procedure, 1 mL of *C. guianensis* flower extracts were added to 500 µL of FC reagent, 1.5 mL of 20% Na_2CO_3, and 6.5 mL of distilled water. The reaction mixtures were incubated for 60 minutes, and OD was measured at 725 nm. Tannic acid (10-100 µg/ml) was used as a standard, and the regression equation of the calibration curve is $y = 0.0043x + 0.0293$ with an R^2 of 0.9372. Results of TTC were reported in terms of Tannic acid equivalents (mg TAE/g) flower extract.

2.4.4 DETERMINATION OF TOTAL FLAVONOID CONTENT

Total flavonoid content (TFC) was quantified by using the aluminum chloride ($AlCl_3$) colorimetric assay (Bao J et al., 2005). In this procedure, 1 mL of *C. guianensis* flower extracts were added to 75 µL of 5% $NaNO_2$ and 1.0 mL of distilled water. The reaction mixtures were incubated for 5 minutes with the addition of 75 µL of 10% $AlCl_3.H_2O$ and 500 µL of 1M NaOH. OD measured at 510 nm. Quercetin (40-200 µg/ml) was used as a standard, and the regression equation of the calibration curve is $y = 0.0045x + 0.02$ with an R^2 of 0.9759. Results of TFC were reported in terms of Quercetin equivalents (mg QE/g) flower extract.

2.5 ANTIOXIDANT ACTIVITIES

2.5.1 DETERMINATION OF TOTAL ANTIOXIDANT ASSAY

Total antioxidant assay (TAA) was estimated by the addition of 0.2 µl of the *C. guianensis* flower extracts (10, 20, 40, 60, 80, and 100 mg/ml) to 2.0 mL of phosphomolybdate reagent containing 28 mM/L NaH_2PO_4, 0.6 M H_2SO_4 and 4 mM $(NH_4)_2MoO_4$ and 1.8 mL water (Prieto et al., 1999). The reaction mixtures were incubated for 90 min at 95 °C and OD was measured at 695 nm. Ascorbic acid (100-500 µg/ml) was used as a standard, and the regression equation of the calibration curve is $y = 0.0006x - 0.003$ with an R^2 of 0.981. Results of TAA were reported in terms of Ascorbic acid equivalents (AAEq mg/ml) flower extract.

2.5.2 DETERMINATION OF FERRIC REDUCING OR ANTIOXIDANT POWER ASSAY

Ferric reducing or antioxidant power (FRAP) assay was estimated by the addition of 0.1 µl of the *C. guianensis* flower extracts (10, 20, 40, 60, 80, and 100 mg/ml) to 3.0 mL of FRAP reagent containing 0.3 M tripyridyltriazine (TPTZ) prepared in 5 ml of 20 mM $FeCl_3$ and 40 mM HCl at pH 3.6 (Benzie IFF et al., 1996). The OD was measured at 593 nm. Ascorbic acid (10-100 mM) was used as a standard, and the regression equation of the calibration curve is $y = 0.0118x + 0.0394$ with an R^2 of 0.9996. Results of FRAP were reported in terms of FRAP Units.

2.5.3 DETERMINATION OF FERRIC REDUCING OR ANTIOXIDANT POWER ASSAY

Reducing power (RP) assay was estimated by the addition of 0.1 µl of the *C. guianensis* flower extracts (10, 20, 40, 60, 80, and 100 mg/ml) to 2.5 ml of phosphate buffer (pH 6.6), 1.0 ml of water (deionized) and 1% 2.5 ml of $K_3[Fe(CN)_6]$ (Quisumbing E. Katha, 1978) and the reaction mixtures were incubated at 50°C for 20 min. To these mixtures 10% TCA and then centrifuged for 10 min at 3000 rpm and after centrifugation, 2.5 ml of the upper layer of the mixture was mixed with 0.5 ml of $FeCl_3$ (0.1%) and 2.5 ml of distilled water. OD was measured at 700 nm. Ascorbic acid (20-100 µg/ml) was used as a standard, and the regression equation of the calibration curve is $y = 0.0063x - 0.0008$ with an R^2 of 0.995. Results of the RP assay were reported in terms of Ascorbic acid equivalents (AAEq mg/ml) flower extract.

2.6 ANTI-INFLAMMATORY ACTIVITIES

2.6.1 HEAT-INDUCED HEMOLYSIS

To establish membrane stabilization as a mechanism, heat-induced hemolysis was performed by preparing two sets at different concentrations (100, 200, 400, 800, and 1000 µg/ml) of flower extracts in a 5 ml isotonic solution containing 154 mM NaCl in 10 mM NaH_2PO_4 (pH 7.4). Positive control was prepared using Diclofenac sodium, whereas negative control was prepared using isotonic solution. To all tubes, 50 µl of 40% RBC suspension was added and by inverting them they were gently mixed. One of the pairs was kept in a water bath at 54°C for 20 minutes and the other in an ice bath at 0-5°C. All tubes were centrifuged at 5000 rpm and the OD was measured at 560 nm (Gandhidasan, R et al., 1991). % inhibition of heat-induced hemolysis (I) was calculated using the following formula: $I = 100 \times (1- (OD_2\text{-}OD_1 /OD_3\text{-}OD_1))$. Where OD_1 is the test sample unheated; OD_2 is the test sample heated and OD_3 is the control sample heated.

2.6.2 EGG ALBUMIN DENATURATION ASSAY

The Egg albumin denaturation assay was performed by the addition of 2.0 mL of flower extracts at different concentrations (100, 200, 400, 800, and 1000 µg/ml) to 0.2 mL of ovalbumin (from freshly harvested eggs) and 2.8 ml phosphate buffer saline (pH 6.4). Distilled water was used as a negative control and Diclofenac sodium as a positive control (Dharmadeva et al., 2018). All mixtures were incubated at 37.2°C for 15 minutes and then heated to 70 °C for 5 minutes in a water bath. OD was measured at 660 nm and the % inhibition of egg albumin denaturation (I) was calculated using the following formula: $I = 100 \times (1- (OD_2\text{-}OD_1 /OD_3\text{-}OD_1))$. Where OD_1 is test sample unheated; OD_2 is test sample heated) and OD_3 is the control sample heated.

III. STATISTICAL ANALYSIS

Results from each experiment were expressed as ± SEM (standard error of the mean), with each experiment being run in triplicate ($N = 3$). Using Microsoft Excel functions a one-way analysis of variance (ANOVA) was conducted followed by a post-hoc Tukey test to identify differences between the means of each sample that were statistically significant at $p \leq 0.05$. Using a linear regression of percent inhibition against the concentration on the concentration-response curve, the IC_{50} values for each sample were determined (Volpe et al., 2014).

IV. RESULTS

4.1 EXTRACTION AND PHYTOCHEMICAL ANALYSIS

To identify the phytochemical components, present in *C. guianensis* flower extracts, qualitative evaluation is necessary. Therefore, a preliminary phytochemical evaluation using standard methods was performed on tannins, cardiac glycosides, phenols, carbohydrates, saponins, flavonoids, alkaloids, and terpenoids. The % of yield (Fig. 1a) of MeOH extracts of male, female, and petals is 9.97 g (2.46%), 0.44 g (1.09%), and 1.32 g (0.29%), respectively. For % of yield, the F statistic is [$F(2, 6) = 24346.8095$, $p \leq 0.05$] and the Tukey test analysis has revealed that the mean % of the obtained weight of MeOH extracts of male (X_1), female (X_2), and petals (X_3) is significantly different from each other with a Q statistic of pair X_1- X_2 ($Q = 192.1074$ and $p = 6.31E\text{-}14$), X_1- X_3 ($Q = 300.8911$ and $p = 6.31E\text{-}14$) and X_2- X_3 ($Q = 108.7836$ and $p = 3.89E\text{-}13$), respectively.

The Preliminary phytochemical investigation (Table 1) of methanolic extracts of male, female, and petals of the flower has revealed the presence of tannins, cardiac glycosides, phenols, carbohydrates, saponins, and alkaloids collectively. However, the test for flavonoids and terpenoids did not show any prominently visible color reactions for any of the three aforementioned MeOH extracts.

Table 1: Qualitative phytochemical analysis of methanolic flower extracts (male, female, and petals) of *Couroupita guianensis*.

Phytochemical constituents	Methanol extract		
	Male	Female	Petals
Tannins	+	+	+
Cardiac Glycosides	+	+	+
Phenols	+	+	+
Carbohydrates	+	+	+
Saponins	+	+	+
Flavonoids	-	-	-
Alkaloids	+	+	+
Terpenoids	-	-	-

Note: + sign, present; - sign, absent

The TPC (Fig 1b) of male, female, and petals MeOH extract is 20.42 ± 2.324, 24.87 ± 5.029, and 27.62 ± 5.516 mg GAE/g extract, respectively. The F-statistic is [$F(2,15) = 0.648$, $p \geq 0.05$]. The TTC (Fig 1c) of male, female, and petals MeOH extract is 67.41 ± 16.032, 50.57 ± 4.760, and 55.21 ± 10.058 mg TAE/g extract, respectively. The F-statistic is [$F(2,15) = 0.596$, $p \geq 0.05$]. Likewise, the TFC (Fig 1d) of male, female, and petals MeOH extract is 9.10 ± 1.13, 14.25 ± 3.669, and 18.60 ± 7.234 mg QE/g extract, respectively. The F-statistic is [$F(2,15) = 1.011$, $p \geq 0.05$]. The Tukey test analysis of TPC, TTC, and TFC has revealed that none of the mean concentrations of MeOH extracts is significantly different from each other. Even though the % of the yield of MeOH extracts of male and female extracts of the flower is remarkably high, compared to petals, it has a high quantity of TPC, TTC, and TFC.

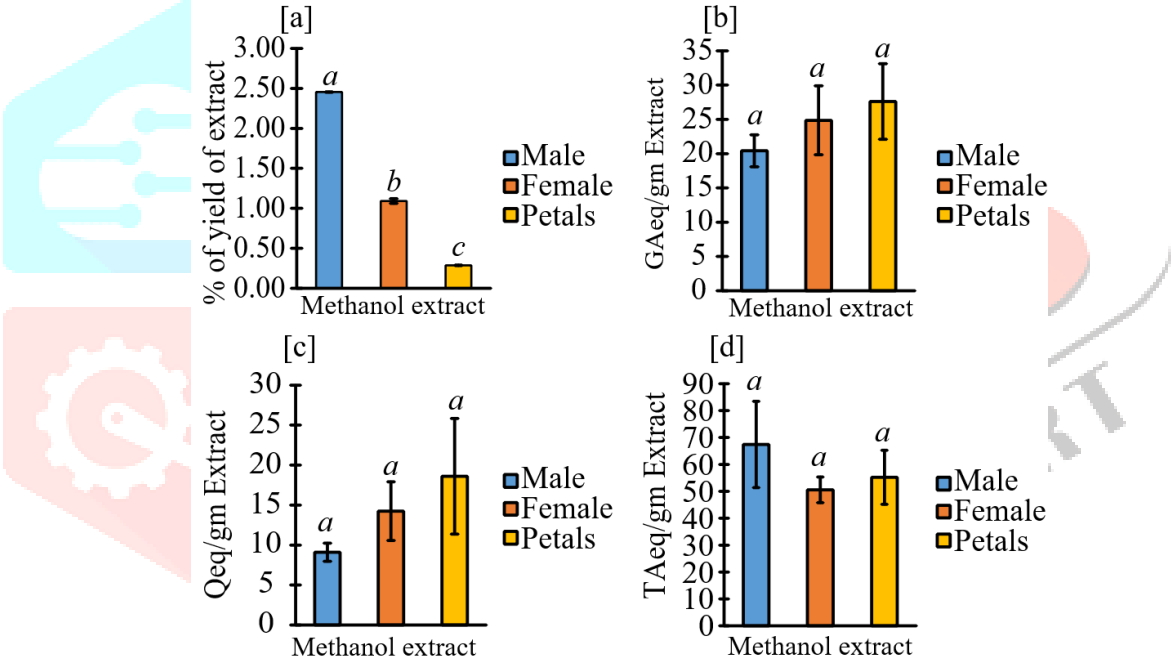

Figure 1. [a] Yield percentage, [b] Total Phenolic Content, [c] Total Flavonoid Content, and [d] Total Tannin Content of methanolic flower extracts of *Couroupita guianensis*. The values shown are the ± SEM of three replications. The Tukey test's mean comparisons across groups and values with distinct letter labels are significantly different at $p \leq 0.05$.

4.2 ANTIOXIDANT ACTIVITIES

In the TA assay (Fig 2a), the F statistic is [$F(3, 20) = 17.843$, $p \leq 0.05$], and the maximum antioxidant activity was reported for MeOH extract of Petals followed by Male and Female extracts, which was 2.98 ± 0.245, 2.15 ± 0.521, and 1.49 ± 0.108 AAEq mg/ml extract, respectively compared to ascorbic acid (standard) which was 0.05 ± 0.014 AAEq mg/ml extract. The Tukey test analysis has revealed that the mean values of AAEq mg/ml extract of male (X_1), female (X_2), and petals (X_3) are significantly different compared to the standard (X_4) with a Q statistic of pair X_1-X_4 ($Q = 7.1807$ and $p = 3.11E-04$), X_2-X_4 ($Q = 4.899$ and $p = 0.01205$) and X_3-X_4 ($Q = 9.9745$ and $p = 4.33E-06$), respectively. Likewise, there is a significant difference between mean pairs of X_2- X_3 with a Q statistic ($Q = 5.0754$ and $p = 9.14E-03$). In the FARP assay (Fig 2b), the F statistic is [$F(3, 20) = 68.205$, $p \leq 0.05$], and the maximum FARP activity was reported for MeOH extract of Petals followed by Male and Female extracts, which was 374.20 ± 7.190, 370.38 ± 2.202, and 366.77 ± 13.81 FRAP Units, respectively compared to standard which was 102.84 ± 28.340 FRAP Units. The Tukey test analysis has revealed that the mean values of FRAP Units of male (X_1), female (X_2), and petals (X_3) are significantly different compared to the standard (X_4) with a Q statistic of pair X_1-X_4 ($Q = 16.5091$ and $p = 1.27E-09$), X_2-X_4 ($Q = 16.2861$ and $p = 1.61E-09$) and X_3- X_4 ($Q = 16.7443$ and $p = 9.91E-10$), respectively.

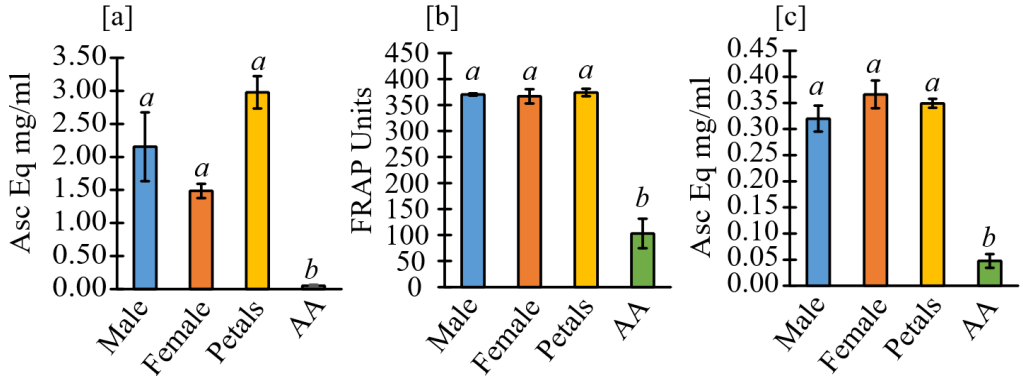

Figure 2. [a] Total antioxidant assay, [b] FRAP assay, and [c] Reducing power assay of methanolic flower extracts of *Couroupita guianensis*. The values shown are the ± SEM of three replications. The Tukey test's mean comparisons across groups and values with distinct letter labels are significantly different at $p \leq 0.05$.

However, there is no significant difference between the mean pairs of any of the flower extracts. In the RP assay (Fig 3c), the F statistic is [$F(3, 20) = 57.19$, $p \leqslant 0.05$], and the maximum FARP activity was reported for MeOH extract of Female followed by Petals and Male extracts, which was 0.37 ± 0.027, 0.35 ± 0.009, and 0.32 ± 0.025 mg/ml extract, respectively compared to the standard which was 0.05 ± 0.013 AAEq mg/ml extract. The Tukey test analysis has revealed that the mean values of AAEq mg/ml extract of male (X_1), female (X_2), and petals (X_3) are significantly different compared to the standard (X_4) with a Q statistic of pair X_1-X_4 (Q = 13.6377 and p = 3.32E-08), X_2-X_4 (Q = 15.9747 and p = 2.26E-09) and X_3-X_4 (Q = 14.9731 and p = 6.90E-09), respectively. Like FRAP activity, there is no significant difference between mean pairs of any of the flower extracts for reducing power capacity.

4.3 ANTI-INFLAMMATORY ACTIVITIES

In the Heat-induced HRBC membrane stabilization assay [$F(19, 40) = 545539.87$, $p \leq 0.05$], amongst the flower extracts in the range of 100-1000 µg/mL, MeOH extract of petals has shown the highest percentage lysis of RBC against heat (24.76 ± 0.023 to 97.75 ± 0.09 %), followed by female extract (26.37 ± 0.024 to 93.25 ± 0.087%), and male (1.29 ± 0.001 to 6.59 ± 0.006) Whereas standard at 100 to 1000 µg/mL shown 18.70 ± 0.0003 to 78.64 ± 0.0033 % activity (Fig 3a). The male, female, petal methanol extracts and standard have IC_{50} values of 9480.79 ± 0.130, 197.73 ± 0.7497, 329.96 ± 0.5234, and 575.40 ± 0.0050 µg/mL, respectively at 95% confidence interval (CI). The obtained low IC_{50} value of Petal MeOH extract compared to other extracts and standard indicates that it is more potent in preventing the RBC from being lysed by heat. Hence, based on the obtained results both MeOH flower extracts (female and petals) of *C. guianensis* showed promising in vitro anti-inflammatory activity.

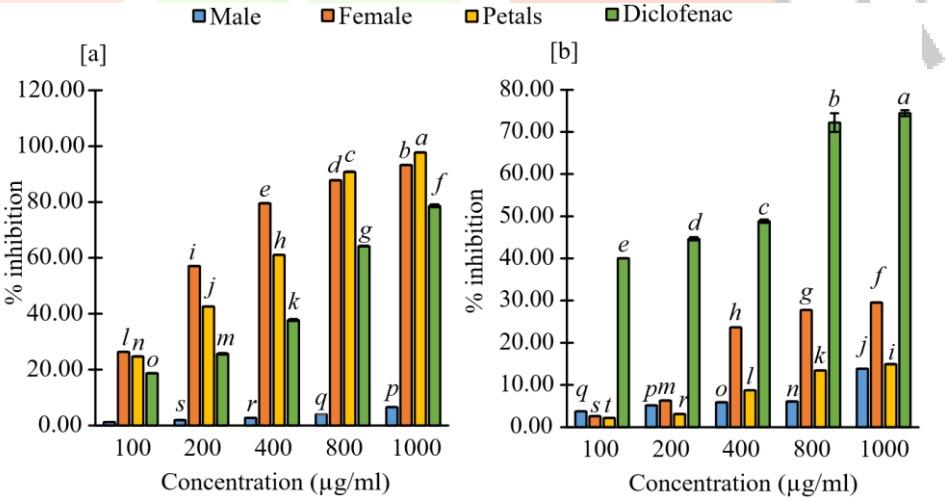

Figure 3. [a] Heat-induced hemolysis and [b] Egg albumin denaturation assay of methanolic flower extracts of *Couroupita guianensis*. The values shown are the ± SEM of three replications. The Tukey test's mean comparisons across groups and values with distinct letter labels are significantly different at $p \leq 0.05$.

Amongst the three types of extracts at a range of 100-1000 µg/mL, the MeOH extract of the Female part of the flower inhibited the denaturation of egg albumin [$F(19, 40) = 601748.18$, $p \leq 0.05$] ranges from 2.59 ± 0.037 to 29.55 ± 0.027%, followed by petals ranges from 2.18 ± 0.040 to 14.94 ± 0.031%, and Male ranges from 3.74 ± 0.035 to 13.85 ± 0.031%. Whereas the standard drug Diclofenac sodium (NSAID) inhibited denaturation by 40.06 ± 0.001 to 74.46 ± 0.0027 % (Fig 3b). The MeOH extracts of male, female, and petals in comparison with the standard have an IC_{50} of 5566.40 ± 4.151, 1579.23 ± 1.253, 3324.94 ± 2.650, and 352.24 ± 0.008 µg/mL, respectively at 95% CI. Hence, in terms of inhibition of protein denaturation, the MeOH of the female extract exhibited higher activity compared to the other two parts of the flower. However, none of the extracts have shown a significant effect on the inhibition of protein denaturation compared to the standard.

4.3 PRINCIPAL COMPONENT ANALYSIS

In principal component analysis (Fig. 4), the total variance across all dimensions with PC_1 and PC_2 axis contributions of 61.01% and 38.99% (PC_1 + PC_2 = 100%). In the given case, there is a very strong positive correlation (r = ≥ 0.5) between TTC and egg albumin denaturation (r = 0.98) concerning phytoconstituents male extract. The TFC and TPCs of petals extract has shown weak positive correlation (r = < 0.5) with FRAP activity (r = 0.47 and r = 0.39), moderate positive correlation (r = ≥ 0.5) with total antioxidant activity (r = 0.5148), and very strong correlation with heat-induced hemolysis (r = 0.9759 and r = 0.95). The TFC of the female extract has a strong positive correlation with reducing power assay (r = 0.63). Whereas TTC of all flower extracts has shown a strong negative correlation (r = < - 0.5) with Reducing power and Heat-induced hemolysis (r = -0.9904 and -0.5695).

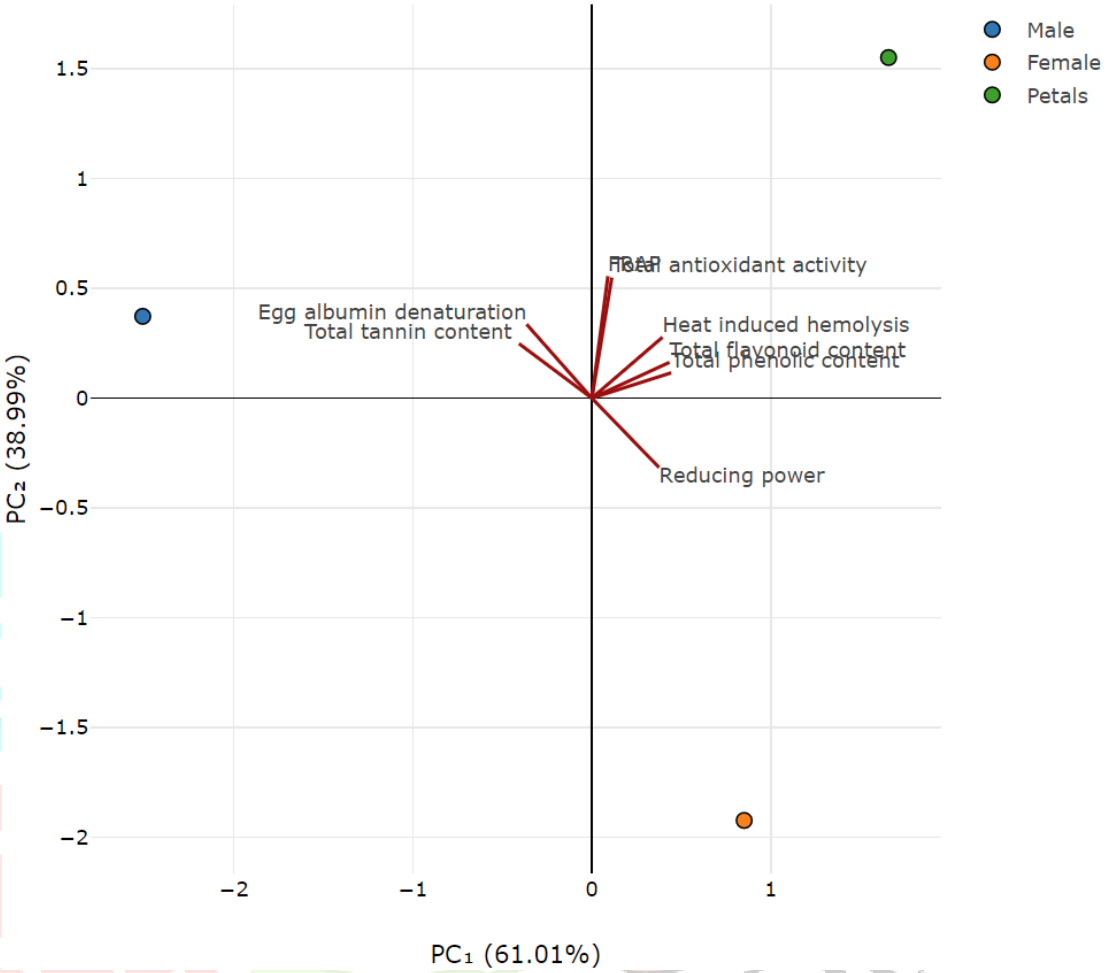

Figure 4. Principal Component Analysis of *Couroupita guianensis* flower extracts, phytochemical components (TPC, TTC, and TFC), antioxidant activities (TAA, FRAP, RP assay), and anti-inflammatory activities (Heat-induced membrane stabilization and Egg albumin denaturation assay).

V. DISCUSSION

It is common knowledge that plants play a significant role in the natural world and are frequently used in the food and pharmaceutical industries. For their biological characteristics, leaves, stems, roots, and bark have been the subject of the most research among the parts of a plant. However, the significance of flowers is not well understood, and they are usually neglected (Bhuvaneswari et al., 2014). Flowers' extracts from plants offer new perspectives on the discovery and development of anti-inflammatory and antioxidant substances. In this work, the phytochemical components of *C. guianensis* flower extract demonstrated antioxidant and anti-inflammatory actions against the reactive oxygen species (ROS) and destabilization of the RBC membrane.

Similar to previous results reported on MeOH extracts from leaves and stems of *C. guianensis*, MeOH extracts of male, female, and petals from *C. guianensis* flowers contain Tannins, Alkaloids, Cardiac Glycosides, Saponins (Kavitha et al., 2011; Pradhan et al., 2009). According to the quantitative results of phytochemical analysis, the MeOH extract of petals contains a significant amount of TPC, TTC, and TFC. In terms of overall antioxidant capacity, reduction of ferric ion (Fe_3^+) to ferrous ion (Fe_2^+) ions, and RBC membrane stabilization (97.75%), phytoconstituents in the MeOH extract of petals have demonstrated excellent action. The flowers of *C. guianensis* have also been said to indicate adequate antioxidant activity.

It is expected that the floral extract's phenolic components are primarily responsible for its antioxidant ability. The phenolic component of petals has anti-inflammatory properties and is effective in the treatment of renal and stomach issues (Logambal et al., 2022). In addition to exhibiting significant free radical scavenging activity, the biosynthesized AgNPs (silver nanoparticles) of *C. guianensis* whole flower extract, flower buds, and CuNPs (copper nanoparticles) petal extract also demonstrated potent antibacterial activity against numerous pathogenic bacterial species (Singh et al., 2021; Kumar, T et al., 2016).

According to Pradhan, D. et al., (2009), methanolic extracts of the whole flower have been shown to have immunostimulant effects on both specific and non-specific immune mechanisms. Isatin (1H-indole-2,3-dione) is an antioxidant with an EC_{50} of 72.80 g/ml & anticancerous with a CC_{50} of 2.94 g/ml against HL60 cell line isolated from the flowers of *C. guianensis*, was reported by Premanathan, et al. in 2012. According to Ramalakshmi et al., (2013), the MeOH floral extract of *C. guianensis* has been shown to have antibacterial action against clinical pathogens like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and fish-borne diseases like *Vibrio mimicus* and *Vibrio harveyi*. As a result, compounds isolated from the flower extracts of the plant *C. guianensis* can be used for disease management as a safe drug to inhibit oxidation, cancer, and wound healing ointments to treat microbial pathogens.

VI. CONCLUSION

The oddity of this study lies in the quantification of phytoconstituents, including numerous antioxidant molecules, using distant parts of the flower. These results are promising enough to undertake bioactive-based fractionation of petal extracts and structural elucidation of active phytoconstituents.

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