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Phytochemical Screening and Anti-Anemic Effect of the Ethanol Extract of *Carica papaya* Ripe Fruit Peel

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ABSTRACT

Anaemia is a common worldwide health issue, especially in impoverished nations. Conventional treatments might be harmful. As a safer option, natural plant-based treatments are being studied more and more. **Objective:** Using both in vitro and in vivo experimental methods, assess the phytochemical profile and anti-anaemic effectiveness of the hydroalcoholic extract (70% ethanol, 30% water) of Carica papaya ripe fruit peel. **Methods:** Using spectrophotometric and chromatographic methods, qualitative and quantitative phytochemical investigations were carried out to detect bioactive chemicals in the extract. After conducting homolysis and antioxidant tests in vitro, phenylhydrazine was used to induce anaemia in female Wistar rats in vivo. Rats were divided into two treatment groups (extract at 150 mg/kg and 300 mg/kg) and control, anaemic, and standard groups at random. Hematological and biochemical parameters, including hemoglobin, RBC count, iron, SOD, and GSH levels, were measured post-intervention. Statistical analyses included ANOVA with post hoc tests for group comparisons. **Results:** Phytochemical screening revealed the presence of flavonoids, phenols, saponins, and alkaloids. Treatment with *Carica papaya* peel extract significantly improved hemoglobin, iron, SOD, and GSH levels compared to anemic controls ($p<0.050$), with efficacy comparable to standard therapy. **Conclusions:** Ethanol extract of *C. papaya* ripe fruit peel demonstrates potent anti-anemic and antioxidant activity, supporting its potential as a natural therapeutic option for anemia management.

INTRODUCTION

Iron deficiency is the leading cause of anemia globally [1]. Current therapies for anemia focus primarily on restoring iron levels through oral or parenteral iron supplementation [2], but are linked with gastrointestinal (GIT) side effects and impaired absorption [3]. The focus on plant-based therapies is growing as an alternative method of managing anemia due to cost-effectiveness and fewer side effects [4]. The different components of the Carica papaya (CP) plant, such as its leaves, seeds, roots, latex, and unripe or ripe fruit, have been utilized to treat diverse ailments [5, 6]. Papaya peel has high levels of phenolic acids, which have antioxidant, antimicrobial, and anti-inflammatory effects [7]. The mineral profile of papaya peel includes iron, calcium, magnesium, potassium, and zinc, which are essential for blood formation and metabolic processes [8]. The presence of vitamin C in the peel further enhances its therapeutic value by promoting iron absorption in the GIT [5]. The ripe fruit peel contains antioxidants that can

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neutralize the free radicals and protect RBC against oxidative stress, which is a typical mechanism in hemolytic anemia [9]. Papaya peel also contains important vitamins A, C, and E, which are synergistic in hematopoiesis [10]. Literature has been written on the nutritional and medicinal value of CP, but limited research has been conducted on the ripe fruit peel.

Few studies have explicitly examined the mature fruit peel, especially in relation to its potential to prevent anaemia, even though *Carica papaya* leaves, seeds, and pulp have been extensively studied. Furthermore, there aren't many thorough studies that use standardized experimental models to assess the phytochemical content and in vivo anti-anaemic benefits of papaya peel extract. Anaemia is still a common medical condition, and current iron treatments have negative side effects and low patient compliance. Investigating safe, economical, and plant-based substitutes is necessary. This study attempts to fill the knowledge gap about the therapeutic potential of *Carica papaya* mature fruit peel in enhancing haematological and biochemical parameters. This study aimed to investigate the potential of the ethanol extract of Carica papaya ripe fruit peel as a natural therapeutic agent for anemia. To compare the anti-anemic effects of the ethanol extract with those of standard treatments and control groups.

METHODS

This experimental study design was conducted in the University of the Punjab, Lahore, from September 2025 to December 2025. The preliminary phytochemical examination of the extract was done by using standard qualitative techniques to determine the existence of different bioactive secondary metabolites. The presence of carbohydrates was determined via Molisch's test. Foam test was used to detect the saponins, and alkaloids were screened with the help of the Mayer reagent. Sample size was determined based on the resource equation method for animal experiments [11]. With five groups and assuming a coefficient of variation of 15%, a sample size of n = 5 per group (total n = 25) was estimated to provide 80% power to detect a 30% difference in primary outcomes at a significance level of $\alpha = 0.05$. This sample size is consistent with previous similar studies evaluating anti-anemic effects of plant extracts in rodent models [12, 13]. The Amino acids were identified by the Ninhydrin test. When the extract was heated, it turned blue in color. Phlobatannins were identified by observing a red precipitate after the addition of one drop of HCL. Quantitative analysis of the secondary metabolites was done using gravimetric analysis, colorimetric method, and High-Performance Liquid Chromatography. The positive control was Triton X-100. After taking ethical approval for the use of animals, Wistar albino female rats (100-150g) were used. Group allocation: Anemia was induced by administering intraperitoneal injections of phenylhydrazine (40 mg/kg) once daily for two consecutive days. Then the animals were divided into six groups (n = 5) and treated for 15 days as follows using a Random Allocation Software, version 2.0. Group 1: Normal control, Group 2: Anemic control (treated with phenylhydrazone 40 mg/kg), Group 3: Standard treatment (ferrous sulfate, 100 mg/kg), Group 4 (Low Dose Extract): Received phenylhydrazine + *Carica papaya* peel extract (150 mg/kg body weight) and Group 5: Received phenylhydrazine + *Carica papaya* peel extract (300 mg/kg body weight). Approximately 1–2 ml of blood was collected from the tail vein after anemia induction. Hemoglobin concentration was estimated using the cyanmethemoglobin method. All Hematological parameters, including RBCs (Hb), hematocrit, PCV, MCH, MCHC, and mean corpuscular volume, were measured. For histopathological examination, the kidney and spleen were isolated and preserved in a 10% formalin solution for further analysis. The peels of Carica papaya were collected, cleaned, shade-dried, and ground into a coarse powder. A hydroalcoholic solution (70% ethanol, 30% water) was used to prepare the extract. A total of 300 g of the fine powdered peel was soaked in 1500 mL of the solution for 72 hours. The mixture was then filtered.

Data were expressed as mean ± standard deviation (SD). Normality of data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. All variables met the assumptions of normality and homogeneity of variance ($p>0.050$), thus parametric tests were applied. Comparisons among groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post hoc test for multiple comparisons. A p-value<0.050 was considered statistically significant. Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The anemia significantly altered most biochemical parameters, with the disease group showing elevated liver enzymes, kidney markers, and oxidative stress changes compared to the control ($p<0.050$) (Table 1).

Table 1: ANOVA for All Biochemical Parameters by Experimental Group

Variables	Group	Mean ± SD	ANOVA p-value
Iron (µg/dL)	Control	126.78 ± 8.42	0.291
	Disease	109.68 ± 7.36	
	Extract 150	118.45 ± 6.89	
	Extract 300	132.67 ± 5.94	

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	Other	80.60 ± 13.02	
	Standard	142.14 ± NA	
SOD (U/mL)	Control	28.24 ± 2.07	0.078
	Disease	164.19 ± 8.37	
	Extract 150	96.45 ± 3.97	
	Extract 300	71.75 ± 6.93	
	Standard	80.87 ± 64.28	
GSH (nmol/mL)	Control	273.55 ± 1.60	0.039
	Disease	20.35 ± 2.24	
	Extract 150	59.89 ± 11.99	
	Extract 300	111.85 ± 11.86	
	Standard	123.04 ± 119.23	
ALT (U/L)	Control	35.00 ± 4.00	0.004
	Disease	79.50 ± 10.61	
	Extract 150	64.33 ± 14.84	
	Extract 300	51.67 ± 10.07	
	Standard	43.67 ± 4.16	
AST (U/L)	Control	60.33 ± 15.28	0.001
	Disease	132.00 ± 12.73	
	Extract 150	92.00 ± 5.57	
	Extract 300	87.00 ± 2.65	
	Standard	95.33 ± 13.80	
ALP (U/L)	Control	114.33 ± 12.66	<0.001
	Disease	364.50 ± 53.03	
	Extract 150	245.67 ± 45.80	
	Extract 300	291.00 ± 28.79	
	Standard	133.00 ± 10.58	
Albumin (g/dL)	Control	3.10 ± 0.50	0.001
	Disease	5.00 ± 0.28	
	Extract 150	4.27 ± 0.59	
	Extract 300	3.03 ± 0.21	
	Standard	3.37 ± 0.06	
Total Protein (g/dL)	Control	5.93 ± 0.15	0.010
	Disease	7.65 ± 0.21	
	Extract 150	6.60 ± 0.36	
	Extract 300	6.00 ± 0.20	
	Standard	6.36 ± 0.74	
Urea (mg/dL)	Control	53.00 ± 5.57	<0.001
	Disease	100.50 ± 4.95	
	Extract 150	78.33 ± 13.05	
	Extract 300	60.67 ± 6.11	
	Standard	67.00 ± 4.58	
Creatinine (mg/dL)	Control	0.47 ± 0.06	0.002
	Disease	1.45 ± 0.35	
	Extract 150	1.00 ± 0.17	
	Extract 300	0.63 ± 0.06	
	Standard	1.03 ± 0.23	

ALT was considerably higher in the illness group than in the control group (p=0.012). Standard therapy versus disease pairwise comparisons revealed statistically significant changes (p<0.050) (Table 2).

Table 2: Tukey HSD Post Hoc Pairwise Comparisons for ALT (U/L) Among Experimental Groups

Group 1	Group 2	Mean Difference	p-value
Control	Disease	-44.5	0.012
Control	Extract 150	-29.3	0.128
Control	Extract 300	-16.7	0.494
Control	Standard	-8.7	0.901
Disease	Extract 150	15.2	0.710
Disease	Extract 300	27.8	0.149
Disease	Standard	35.8	0.033
Extract 150	Extract 300	12.7	0.794
Extract 150	Standard	20.7	0.304
Extract 300	Standard	8.0	0.931

The standard treatment group had ALP levels closest to the control, showing significant differences when compared with both extract groups (p=0.002 and p<0.001) (Table 3).

Table 3: Tukey HSD Post Hoc Pairwise Comparisons for ALP (U/L) among Experimental Groups

Group 1	Group 2	Mean Difference	p-value
Control	Disease	-261.0	<0.001
Control	Extract 150	-131.33	0.001
Control	Extract 300	-176.67	<0.001
Control	Standard	-11.33	0.997
Disease	Extract 150	129.67	0.001
Disease	Extract 300	84.33	0.017
Disease	Standard	249.67	0.000
Extract 150	Extract300	-45.33	0.441
Extract 150	Standard	120.0	0.002
Extract 300	Standard	165.33	<0.001

The disease group had significantly higher urea compared to the control group (p=0.001). Both the 300 mg/kg extract (p=0.002) and the standard treatment (p=0.007) significantly reduced urea, while the 150 mg/kg extract showed a near-significant reduction (p=0.072) (Table 4).

Table 4: Tukey HSD Post Hoc Pairwise Comparisons for Urea (mg/dL) among Experimental Groups

Group 1	Group 2	Mean Difference	p-value
Control	Disease	-47.50	0.001
Control	Extract 150	-25.33	0.045
Control	Extract 300	-7.33	0.888
Control	Standard	-14.00	0.537
Disease	Extract 150	22.17	0.072
Disease	Extract 300	40.17	0.002
Disease	Standard	33.50	0.007
Extract 150	Extract 300	18.00	0.267
Extract 150	Standard	11.33	0.678
Extract 300	Standard	-6.67	0.912

Significant differences were found between the control group and all other groups (p<0.050). No statistically significant differences were observed among other groups

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(p>0.050), indicating that treatments improved GSH compared to disease alone, but did not fully restore levels to those of the control group (Table 5).

Table 5: Tukey HSD Post Hoc Pairwise Comparisons for GSH (nmol/mL) Among Experimental

Group 1	Group 2	Mean Difference	p-value
Control	Disease	253.2	0.001
Control	Extract 150	213.6	0.002
Control	Extract 300	161.7	0.011
Control	Standard	150.5	0.009
Disease	Extract 150	-39.6	0.940
Disease	Extract 300	-91.5	0.556
Disease	Standard	-102.7	0.426
Extract 150	Extract 300	-51.9	0.758
Extract 150	Standard	-63.1	0.641
Extract 300	Standard	-11.2	0.999

The study illustrates the comparative mean values of glutathione (GSH). Treatment groups (150 mg/kg and 300 mg/kg) increased GSH dose-dependently, suggesting a partial restoration of antioxidant capacity. SOD activity, a critical enzymatic antioxidant measure, was highest in the Disease group (164.2 U/L) and lowest in the Control group (Figure 1).

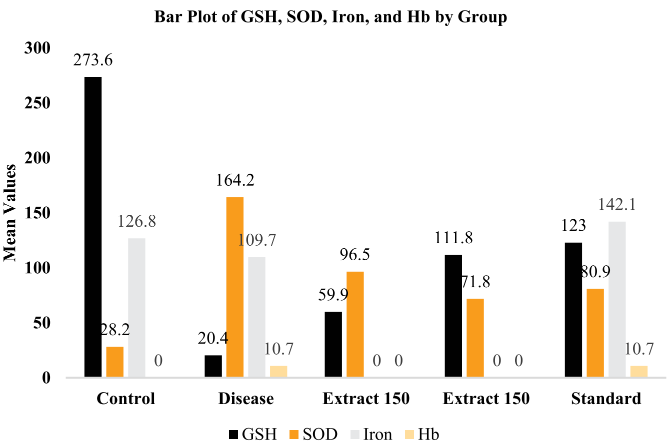

Figure 1: Bar Plot of GSH, SOD, Iron, and Hb by Group

The Disease group displayed significant elevations in ALT (79.5 U/L), AST (132.0 U/L), and ALP (364.5 U/L) relative to the Control group. Extract treatment groups and the Standard group demonstrated lower enzymatic levels, indicating partial hepatic protection. The disease group had higher quantities of albumin (5.0 g/dL), total protein (7.65 g/dL), urea, and creatinine (100.5 mg/dL and 1.45 mg/dL, respectively) than the control group (Table 2).

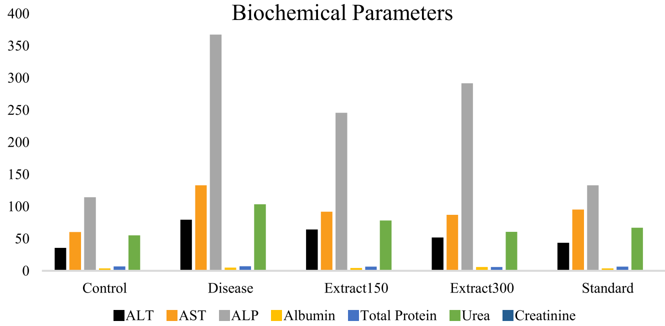

Figure 2: ALT, AST, ALP, Albumin, Total Protein, Urea, and Creatinine by Group

DISCUSSION

Assessing the phytochemical composition and anti-anaemic qualities of an ethanol extract of the mature fruit peel of the *C. papaya* was the aim of this investigation. In the current investigation, the illness group had lower iron levels than the control group. This pattern is in line with the recognized effects of phenylhydrazine, which mainly cause anaemia by oxidative processes that damage haemoglobin and erythrocyte membranes [14, 15]. Glutathione (GSH) levels were significantly lower in the illness group in the current investigation, suggesting that this important intracellular antioxidant was being depleted. This result is in line with a study showing that the injection of phenylhydrazine causes considerable GSH depletion and oxidative stress. Iron concentrations increased after treatment with *C. papaya* peel extract, particularly at 300 mg/kg, indicating that it may play a part in improving iron availability or absorption. This is consistent with research showing that papaya leaf extract considerably raised blood iron levels in anaemic rats [12]. In line with findings that phenylhydrazone-induced anaemia increased liver enzymes, the illness group in the current investigation had significantly higher levels of ALT, AST, and ALP, indicating hepatocellular damage [16–18]. These enzyme levels were lowered by treatment with C. papaya peel extract, with the most noticeable effects occurring at 300 mg/kg. Although the extract-treated groups' serum iron levels were quantitatively greater than those of the illness group, the overall ANOVA did not show a statistically significant difference between the groups (p=0.291). This implies that either the sample size was too small to detect a slight effect, or the extract might not have a significant impact on iron status under the given experimental settings. To fully understand how *C. papaya* peel extract affects iron metabolism, more research using bigger sample sizes is required. Further, Treatment with the 300 mg/kg extract and the standard drug significantly lowered urea and creatinine levels compared to the disease group, indicating nephroprotection. Similarly reported that papaya leaf extract preserved kidney function in oxidative injury models, due to the plant's antioxidant and anti-

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inflammatory phytochemicals [19]. In the current study, 300 mg/kg extract performed comparably to ferrous sulfate. This can be used as a plant-based alternative in anemia management. The lower dose (150 mg/kg) showed partial benefits, indicating a dose-dependent effect. These findings are compatible with the previous study, which evaluated the efficacy of CP in treating IDA [20]. Clinical study reported that patients treated with more concentrated CP herbal extract and 30°C potency (a highly diluted homeopathic preparation experienced a positive shift in their blood levels in terms of Hb increased by 0.6 gm/dl and 0.4 gm/dl, respectively. The 30C potency demonstrated a more efficient ability in treating IDA [13].
The study relied on a relatively small cohort of experimental animals, and only female Wistar rats were utilized; the findings may not account for sex-based physiological variations. long-term safety profile was not considered. The potential benefits of other extraction methods remain unexplored, and the lack of human clinical trials requires further validation in more diverse and long-term studies. Future investigations should prioritize larger, gender-diverse animal cohorts and long-term treatment durations to better establish the extract's comprehensive safety and efficacy profiles.

CONCLUSION

In phenylhydrazine-induced anaemic rats, this study showed that the ethanol extract of Carica papaya mature fruit peel had important anti-anaemic, antioxidant, hepatoprotective, and nephroprotective qualities. In correcting abnormal biochemical markers, the higher dosage (300 mg/kg) was more beneficial and frequently showed effectiveness comparable to typical ferrous sulfate therapy. To prove its effectiveness and safety in humans, more clinical studies are necessary.

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Authors' Contribution

Conceptualization: RF
Methodology: RF, MSU, NI
Formal analysis: SAS
Writing and Drafting: SAS, SA, SKA
Review and Editing: RF, SAS, MSU, SA, SKA, MNA, NI

All authors approved the final manuscript and take responsibility for the integrity of the work.

Conflicts of Interest

All the authors declare no conflict of interest.

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