ISSN No: 0976-5921

Research Article

Unveiling the Physicochemical Properties, Mineral Profile and Hemo-Protective Potential of *Acacia Suma*

Nikita N Kanbarkar[1]*, Shubhada Malave[2], Sangamesh Halli[2], Vidya Surywanshi[2], Prajwal Hiremath[2], Sumitra Hulyal[2]

1. Associate Professor, Pharmacology Department, Rani Chennamma College of Pharmacy, Vaibhav Nagar, Belagavi, Karnataka. India.
2. Final year B Pharm Students, Rani Chennamma College of Pharmacy, Vaibhav Nagar, Belagavi, Karnataka, India.

Received: 14-05-2025 Accepted: 30-11-2025 Published: 31-12-2025

Abstract

Anaemia is condition when haemoglobin (Hgb) level is <13.0gm/dL in men and <12.0gm/dL in female. In women blood disorders like anaemia, menstruation problems, miscarriage, foetus death, ischemia and stroke all are life threatening problems. The *Acacia suma* has been reported in Ayurvedic Pharmacopeia of India for traditional role in treatment of blood disorders. The hydroalcoholic extract yield was 9.75% and shown presence of phenols, flavonoids and tannins. The hemolytic assay and osmotic fragility assay were performed and percent hemolysis was analysed as Mean ± SD. Further, mineral content of the extract was determined using flame Atomic Absorption Spectrometry. In phytochemical screening the hydroalcoholic extract of *Acacia suma* shown presence of carbohydrate, proteins, amino acid, steroids, saponin, flavonoids, alkaloids, tannins and phenols. At concentration 250µl/ml, 150µl/ml and 50µl/ml the extract revealed 24.53 ± 3.92 %, 30.63 ± 4.18 % and 54.66 ± 8.92 percent hemolysis respectively. In mineral calcium was found highest among the elements (390.58 ± 7.39 ppm) followed by zinc (26.488 ± 0.32 ppm) and iron (25.84 ± 0.79 ppm). As concentration increases, extract exhibited significant hemo-protective potential and it could be an effective medicine for the treatment of iron deficiency anemia and hemolytic anemia.

Keywords: *Acacia suma*, Anaemia, Hemolytic anaemia, Iron deficiency anaemia, Zinc deficiency.

Access this article online

Website: https://ijam.co.in

DOI: https://doi.org/10.47552/ijam.v16i4.6155

Introduction

Blood is a connective tissue and plays important role in transporting nutrients throughout the body, helps in exchange of gases mainly oxygen and carbon dioxide, act as defence system in pathogenic condition, removes out metabolic waste from the body and with the help of clotting mechanism controls heavy bleeding in severe injuries(1). Women's are more prone to have blood disorders including anaemia, menstruation problems, miscarriage, foetus death, thrombosis in placenta, ischemia and stroke(2). Anaemia is one of the leading blood disorder among the women's, WHO factsheet published on May 2023 says that globally 40% of children's at the age 6-59 months, 37% of pregnant women and 30% non-pregnant women of 15-49 years of age are suffering from anaemia https://www.who.int/news-room/fact-sheets/detail/anaemia (09/03/2025; 11:25).

The bone marrow disfunction(3), kidney disfunction (4), liver disease(5), lactation during pregnancy(6) and deficiency of minerals and vitamins may cause anaemia(7). When haemoglobin (Hgb) reaches to less than 13.5 gm/dL in men and 12.0 gm/dL in female it elevates anaemia(8). Anaemia in women is often due to deficiency of iron, zinc and B12 vitamin deficiency. Several types of anaemia affecting humans are iron deficiency anaemia, megaloblastic anaemia, sickle cell anaemia and haemolytic anaemia. Deficiency of iron and zinc; which are responsible for metabolism, neuronal function and immune system(9)(10) may contribute to anaemia.

The *Acacia suma* (Roxb.) is a ayurvedic medicine belongs to family Fabaceae, API (Ayurvedic Pharmacopeia of India) mentioned traditional use of *Acacia suma* in Skin infections, Mouth diseases, Blood related diseases, Diabetes and Obesity(11)(12). Also the 'Acacia' species are reported as good source of minerals like calcium, iron, zinc, copper and magnesium (13). Few species of Acacia are reported for anti-anaemic potential like *Acacia senegel*(14), *Acacia nilotica* (15) and *Acacia Hydaspica* (16). A researcher Widowati R. *et. al*. 2024, reported consumption of honey obtained from *Acacia* species promotes elevation in haemoglobin in pregnant women(17). Considering the broad medicinal properties of *Acacia suma*, the present study was designed to screen anti-haemolytic property of hydroalcoholic extract of *Acacia suma* heartwood by *in-vitro* method.

Materials and Methods

Collection and authentication of plant material

The heartwood of *Acacia suma* was procured and authenticated by Dr K. Madhava Chetty, Plant taxonomist, Assistant professor, Department of Botany, Shri Venkateshwara University, Tirupati – 02; plant accession number 0661.

*** Corresponding Author:**
Nikita N Kanbarkar
Associate professor; Pharmacology Department,
Rani Chennamma College of Pharmacy,
Belagavi– 590010, Karnataka, India.
Email Id: nikkanbarkar@gmail.com

Extract preparation

The *Acacia suma* heartwood powder was subjected for maceration for 7 days using ethanol and water in a ratio (70:30) to obtained hydroalcoholic extract (18).

Ash value determination

Determination of total ash

The coarse and dried form of 2 g of test material was weighed in a silica crucible and placed in the incinerator at 500°C till it converts into ash. The crucible was weighed again after cooling and total ash content (%) was calculated (19).

Total ash = Ash weight / initial weight of powder (g) × 100

Determination acid insoluble ash

The ash obtained from total ash was boiled for 5 minutes using 45 ml of dilute HCl. The mixture was filtered through ash-less filter paper, and the resulting residue was washed with hot water. The filter paper was ignited and weight recorded.

Acid insoluble ash (%) = Acid insoluble ash weight / initial weight of powder (g) × 100

Determination water-soluble ash

Similarly, the ash obtained from total ash was boiled for 5 min. using 45 ml of distilled water. The mixture was filtered using ash-less filter paper and obtained residue was washed with hot water. After complete drying the filter paper was ignited and weight was noted. The water-soluble ash (%) was calculated.

Water soluble ash = water soluble ash weight / initial weight of powder (g) × 100

Determination moisture content

Initially a glass-stopper bottle weight was noted and 2 gm of test material was placed in the bottle, The bottle loaded with test material was placed into the oven. The material was dried till to get constant weight readings. After cooling, bottles final weight was noted to calculate percent moisture content (20).

Moisture content (%) = Loss in weight / weight of powder (g) × 100

Determination of extractive values

Alcohol soluble extractive

The 2.5 g of coarse powder of heartwood of *Acacia suma* placed in 250ml of conical flask for macerated with 100 ml of ethanol for 24 hours. After shaking frequently for 6 h the flask was allowed to stand for 18 hours. It was then filtered and 25 ml of the filtrate was evaporated to dryness at 105±1°C and weighed (21). The percent alcohol-soluble extractive was calculated.

Water soluble extractive

The 2.5 g of coarse powder of heartwood of *Acacia suma* placed in 250ml of conical flask for macerated with 100 ml of ethanol for 24 hours. After shaking frequently for 6 h the flask was allowed to stand for 18 hours. It was then filtered and 25 ml of the filtrate was evaporated to dryness, at 105 ± 1°C and weighed. The percent water-soluble extractives was calculated (21).

Determination of pH

The pH value of aqueous solution of plant material (1% w/v) was determined.

Fluorescence analysis

Fluorescence analysis was carried out by mixing the crude powder of test material with various reagents like HCl, H^2SO^4, HNO^3, NaOH and Ammonia. The presence of fluorescence was observed under UV chamber at 254 nm and 366 nm (22).

Phytochemical evaluation

Phytochemical screening of hydroalcoholic extract of *Acacia suma* heartwood was performed to confirm the presence of various phytoconstituents(22).

Blood sample collection

The blood sample (10ml) was procured from "Belgaum Blood Centre" blood bank located at Ayodhya Nagar, behind Vijaya hospital, Old P.B. Road, Belagavi, Karnataka India. The assays were performed in tissue culture laboratory at KAHER's Basic Science Research Centre (BSRC), Nehru Nagar Belagavi, Karnataka India.

In-vitro hemolytic assay

The blood sample was collected in heparin tube and centrifuged at 1700 rpm for 5 min. supernatant was removed and rinsed erythrocytes by adding 2 mL of PBS (7 pH). Again, centrifuged at 1700 rpm for 5 min. This step was repeated for three times until a clear supernatant was obtained. In ratio 1:100 erythrocyte pallet was diluted with PBS to obtained 1% erythrocyte suspension. Mixed 50µl (1%) erythrocyte suspension with 50 µL of extract (50µg/ml, 150 µg/ml and 250 µg/ml) in a 96-well plate. 10% Triton X-100 was used as a positive control and PBS (7 pH) as a negative control. The 96-well plate was incubated at 37 °C for one hour and absorbance measured at 405 nm.; % hemolysis was calculated using following formula (16) (23).

Hemolysis percentage- OD of test – OD of negative control / OD of positive control-OD of negative control x 100

Osmotic fragility assay

Hypotonic saline solution (7.4 pH) was prepared with different NaCl concentration (0.2%-0.85%). 10µl sample of RBC was added to 1990µl of each hypotonic saline solution (control). 10µl of extract were added to 1980µl of each concentration of hypotonic solution and added 10µl RBC suspension. The mixture was allowed to stand for one hour at room temperature. Further centrifuged at 5000 rpm for 5min. and absorbance was measured at 540 nm.

Osmotic fragility (24) - OD of test – OD of negative control / OD of positive control – OD of negative control x 100

Mineral Profile: Determination of iron, zinc and calcium content of extract

Material and reagents

Hydroalcoholic extract of *Acacia suma* (5 gm), AAS standards for iron, zinc and calcium, Nitric acid, Hydrochloric acid, Hydrogen peroxide (5%), Milli-Q type-I ultra-pure water, crucible, micropipettes and reagent bottle. The minerals determination was performed at KAHER's Food and Micronutrient Analysis Laboratory J. N. Medical College Belagavi, Karnataka; using flame Atomic Absorption Spectrometry (AAS).

Standard preparation

Standard stock solution of iron, zinc and calcium were prepared using standard AAS reagents for iron, zinc and calcium in nitric acid.

Preparation of working standards

Working standards for iron, zinc and calcium was prepared by withdrawing 100 µl solution from standard stock in 100 ml of volumetric flask and 2% of HNO^3 was added up to the mark to achieve final concentration and calibration curve was obtained.

Test sample preparation

The muffle furnace heated at 900 ºC, initial weight of empty crucible was measured. The dried, extract of *Acacia suma* (5.0 gm) added into the crucible. The crucible placed into the pre-heated muffle furnace to obtained ash. Final weight of the crucible noted and 5 ml of aqua regia (8ml of 70% nitric acid and 24ml of 36% hydrochloric acid) added drop wise; mixed it well using glass rod to dissolved the ash completely. Further, mixture was filtered and filtrate collected in 50 ml volumetric flask, volume adjusted up to the mark with 2% nitric acid and the final mixture was used for the determination of iron, zinc and calcium by Atomic Absorption Spectrometry (AAS)(25).

Results

Evaluation of physicochemical parameters

Table 1: Physicochemical analysis of crude powder of heartwood of *Acacia suma*

Parameters	Result
Color	Yellowish-brown
Odor	Characteristic
Taste	Bitter
Yield of extract	9.75%
Water soluble extractive	7.2 ± 0.8
Alcohol soluble extractive	1.06 ± 0.61
Total ash	3.33 ± 0.76
Acid insoluble ash	1.83 ± 0.58
Water-soluble ash	3.16 ± 1.04
Loss on drying	5.5 ± 0.5
pH	5.8 ± 0.3

Figure 1: *Acacia suma* heartwood extract

The crude powder of *Acacia suma* was tested for physiochemical properties and found that the % water soluble of extractive was higher than the alcohol soluble extractive. Further, the yield of hydroalcoholic extract of found 9.75% (Table 1). A fine brown colour powder of hydroalcoholic extract of *Acacia suma* was obtained after lyophilization and was stored in air tight glass bottle (Figure 1).

Fluorescence Analysis

Table 2: Fluorescence analysis of crude powder of *Acacia suma* heartwood

Reagents	UV light 254 nm	Visible light 366 nm
Powder	Whitish	Pale white
Powder + water	Whitish-yellow	Pale white
Powder + HCl	Greenish-yellow	Yellowish- green
Powder + H_2SO_4	Greenish-yellow	Brownish-yellow
Powder + HNO_3	Greenish-yellow	Greenish-yellow
Powder + KOH	Yellowish	Pale-Yellowish
powder + NaOH	Yellowish-brown	Yellowish
Powder + Ammonia	Greenish-yellow	Pale yellow

The crude powder of *Acacia suma* was further evaluated for presence of fluorescence using various reagents such as HCl, H^2SO^4, HNO^3, KOH, NaOH and Ammonia. In presence of HNO^3 powder of *Acacia suma* shown greenish-yellow colour fluorescence at 366 nm (Table 2).

Phytochemical screening of extract

Table 3: Phytochemical screening of HEA of *Acacia suma*

Phytochemical test	Inference
Carbohydrates	+
Proteins	+
Amino acids	+
Steroids	+
Saponin Glycosides	++
Cardiac Glycosides	-
Anthraquinone Glycosides	-
Flavonoids	++
Alkaloids	++
Tannins and Phenolic Compounds	++

The phytochemical screening of hydroalcoholic extract of *Acacia suma* has revealed the presence of saponin, flavonoids, alkaloids and tannins; which could be responsible for hemo-protective property of extract (Table 3).

Hemolytic assay

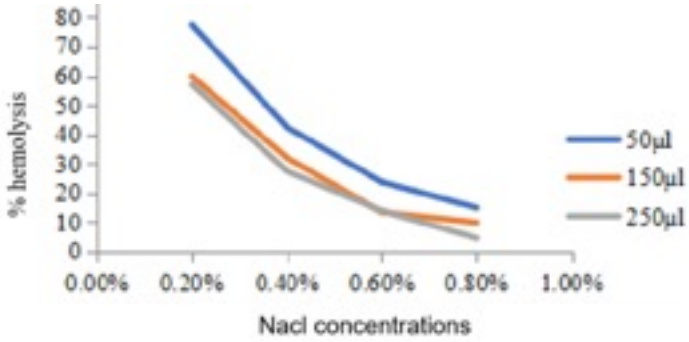
Table 4: Effect of *Acacia suma* extract on hemolysis

S. No.	50µl/ml	150µl/ml	250µl/ml
1	54.75%	26.01%	20.6%
2	44.56%	25.9%	28.6%
3	70.51%	36.3%	30.2%
4	48.82%	29.7%	22.9%
5	56.98%	33.1%	21.6%
6	52.34%	32.8%	23.3%
Mean ± SD (%)	54.66 ± 8.92	30.63 ± 4.18	24.53 ± 3.92

The extract shown hemo-protective property at various concentrations 50µl/ml, 150µl/ml and 250µl/ml as concentration dependent manner however the % hemolysis was found as 54.66 ± 8.92, 30.63 ± 4.18 and 24.53 ± 3.92 respectively (Table 4).

Osmotic fragility assay

Figure 2: Percent hemolysis in different salt concentration



The osmotic fragility of RBC observed under various hypotonic salt concentrations (0.2-0.8). As the concentration of NaCl increases from 0.2% to 0.8% the percent hemolysis has decreased. In 0.8 % salt solution reduced hemolysis was observed up to 4.53 %, 9.77 % and 14.85 % at 250µl/ml, 150µl/ml and 50µl/ml concentration of extract respectively (Figure 2).

Mineral profile of *Acacia suma* extract

Table 5: Analytical conditions for Fe, Zn and Ca

Element	Integration	Replication	R square	Wavelength (nm)	Slit (nm)
Fe	3 Seconds	3	0.999	248.3	0.2
Zn	3 Seconds	3	0.991	213.9	1.0
Ca	3 Seconds	3	0.997	422.7	0.5

The minerals from *Acacia suma* extract were determined using Atomic Absorption Spectrometry and analytical conditions are represented in (Table 5).

Figure 3: Calibration curve for Iron

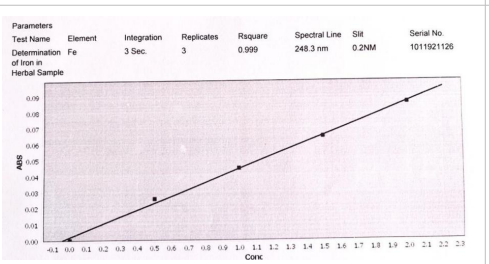


Figure 3 represents the calibration curve o with R square value 0.999 at 248.3 nm.

Figure 4: Calibration curve for Zinc

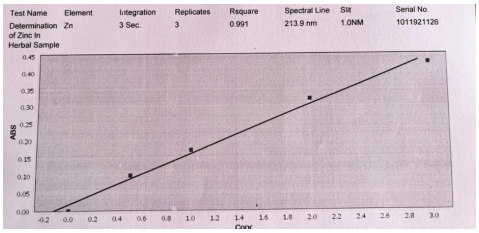


Figure 4 represents the calibration curve o with R square value 0.991 at 213.9 nm.

Figure 5: Calibration curve for Calciu

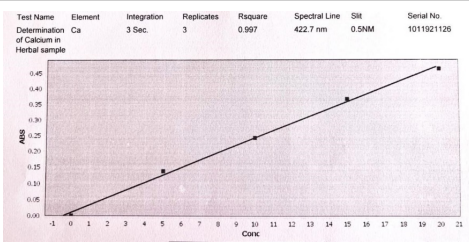


Figure 5 represents the calibration cur calcium with R square value 0.997 at 422.7 n

The graphical representation of calibration curve of Fe, Zn and Ca shown in (Figure 3), (Figure 4) and (Figure 5) respectively. The calcium was found significantly high among the elements (390.58 ± 7.39 ppm) followed by zinc (26.488 ± 0.32 ppm) and iron (25.84 ± 0.79 ppm).

Discussion

Evaluation of physicochemical parameters of crude drug is important to confirm the purity and to obtained clear species validation both by qualitatively and quantitatively(26). The results of physicochemical parameters for *Acacia suma* heartwood (Table 1) are found in compliance with those values reported in Ayurvedic Pharmacopeia of India, (Vol. 5). The fluorescence analysis of crude material of *Acacia suma* heartwood was performed with HCl, HNO^3, H^2SO^4, NaOH, and Ammonia and observed under UV light and visible light (Table 2). Fluorescence testing is one of the parameter to check the authenticity of the crude drug which helps in identifying the intensity of phytochemicals wavelength present in the drug(27). The drug has shown fluorescence in presence of HNO^3 at wavelength 366 nm.

In phytochemical screening hydroalcoholic extract of *Acacia suma* heartwood revealed presence of carbohydrate, proteins, amino acid, steroids, saponin, flavonoids, alkaloids, tannins and phenols whereas cardiac and anthraquinone glycosides were found absent (Table 3). The epigallocatechin, fisetin and quercetin were found in extract(18) could be responsible for the significant RBC protective potential. as it has been reported that flavonoids possess membrane protective efficiency to the RBC(28).

The decreased production of RBC is responsible for hypoxia leads to inflammation and necrosis of tissue. Deficiency of minerals like iron, zinc and B12 vitamin promote hemolysis or decreases hemoglobin content (29). The ruptured RBC membrane due to oxidative stress exerted on membrane, radiation or lack of anti-oxidants can cause hemolysis(16). The extract concentration 250µl/ml, 150µl/ml and 50µl/ml shown 24.53 ± 3.92 %, 30.63 ± 4.18 % and 54.66 ± 8.92 % haemolysis respectively (Table 4).

The capacity of erythrocytes to resist hemolysis was tested using 2-8% of hypotonic salt solution; and observed that with 2 % salt solution hemolysis was increased by 56.95 %, 59.72 % and 77.24% in presence of 250µl/ml, 150µl/ml and 50µl/ml of extract concentration respectively. As the percent NaCl increases from 0.2% to 0.8% the percent hemolysis has decreased in three concentrations of extract. In 0.8 % salt solution reduced hemolysis was observed up to 4.53 %, 9.77 % and 14.85 % at 250µl/ml, 150µl/ml and 50µl/ml concentration of extract respectively (Figure 2). The spherocytosis is the abnormality leads to red blood cell membrane damage and reduced half-life of RBC and contribute in anemic condition (30).

The analytical conditions are mentioned (Table 5) for the estimation of iron, zinc and calcium using Atomic Absorption Spectrometry. The graphs obtained for Zinc (Figure 3), Iron (Figure 4) and Calcium (Figure 5) against standard reagents of

AAS for Zn, Fe and Ca at wavelength 213.9, 248.3, and 422.7nm respectively. The amount of calcium was found highest among the elements (390.58 ± 7.39 ppm) followed by zinc (26.488 ± 0.32 ppm) and iron (25.84 ± 0.79 ppm) in the extract. Deficiency of zinc and iron with folate, vitamin A, B^6 and B^{12} deficiency is associated with anaemia and may leads to iron deficiency anaemia (31) (32). Furthermore, the hydroalcoholic extract of *Acacia suma* previously been evaluated for significant anti-oxidant property with quantitative estimation of phenol 571.49±27.3 mg (GAE/g), tannins 165.9±8.6 mg (TAE/g) and flavonoids 92.3±15.2 mg (QE/g)(18).

The *Acacia suma* extract was validated for the presence of epigallocatechin, fisetin and quercetin by simultaneous estimation using HPTLC(33). Antioxidants are the agents which protects RBC from oxidative damage in iron deficiency anaemia(24). This in-vitro investigation suggests that *Acacia suma* extract has strong potential to mitigate hemolytic anaemia and iron deficiency anaemia.

Conclusion

The study includes systemic evaluation of physicochemical parameters to supports the authenticity of the drug along with phytochemical screening which proves availability of primary and secondary phytochemicals could be responsible for hemo-protective activity. Considering the anti-hemolytic potential of the extract, effective investigation required for in-detailed anti-anaemic role of *Acacia suma* by well-established *in-vivo* models particularly for the flavonoids such as fisetin, quercetin and epigallocatechin.

Financial support Rani Chennamma College of Pharmacy, Belagavi, Karnataka-India.

Conflict of interest No conflict of interest

Acknowledgement: The authors are thankful to management, director and principal of Rani Chennamma College of Pharmacy, Belagavi. Karnataka.

The authors are thankful to Mrs. Dhanashree Patil Research Associate Grade I, KAHER's BSRC Belagavi. and Mrs. Shital Swapnil Patil; Junior Analyst, KAHER's Food and Micronutrient Analysis Laboratory J. N. M. C. Belagavi Karnataka.

References

1. Mann K. Biochemistry and Physiology of Blood Coagulation. Thromb Haemost. 1999;82(08);165–74.
2. Ebrahim, Shahul H. Blood Disorders Among Women. Am J Prev Med. April,2010;38(4);459–67.
3. Ruifrok WPT, Qian C, Silljé HHW, Van Goor H, Van Veldhuisen DJ, Van Gilst WH, et al. Heart failure-associated anemia: bone marrow dysfunction and response to erythropoietin. J Mol Med. April,2011;89(4);377–87.
4. Ezenwaka CE, Jones-LeCointe A, Nwagbara E, Seales D, Okali F. Anaemia and kidney dysfunction in Caribbean Type 2 diabetic patients. Cardiovasc Diabetol. August,2008,7(1):25.
5. Gonzalez-Casas R, Jones EA, Moreno-Otero R. Spectrum of anemia associated with chronic liver disease. WJG. October,2009;15(37);4653.
6. Basrowi RW, Zulfiqqar A, Sitorus NL. Anemia in Breastfeeding Women and Its Impact on Offspring's Health in Indonesia: A Narrative Review. Nutrients. April,2024;16(9):1285.
7. Sim JJ, Lac PT, Liu ILA, Meguerditchian SO, Kumar VA, Kujubu DA, et al. Vitamin D deficiency and anemia: a cross-sectional study. Ann Hematol. May,2010;89(5);447–52.
8. Turner J, Parsi M, Badireddy M. Anemia. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025.
9. Beard JL. Iron Biology in Immune Function, Muscle Metabolism and Neuronal Functioning. J Nutr. February,2001;131(2);568S-580S.
10. Kiouri DP, Tsoupra E, Peana M, Perlepes SP, Stefanidou ME, Chasapis CT. Multifunctional role of zinc in human health: an update. EXCLI Journal. August,2023:1611-2156.
11. Department of AYUSH, Government of India. Ayurvedic Pharmacopoeia of India. Vol. 5. New Dehli, Government of India Ministry of Health and Family Welfare.
12. Vaidyaratnam P S. Indian Medicinal Plants. Vol. 1. Arya VaidhyaSala, Kottakkal; 1994. 30–31p.
13. Pedro SI, Gonçalves J, Horta C, Gonçalves JC, Gominho J, Gallardo E, et al. A Systematic Analysis of Nutritional and Mineral Composition and Toxicity in *Acacia Species* Leaves. Appl Sci. October,2024;14(20);9437.
14. Kaddam LA, Kaddam AS. Effect of Gum Arabic (*Acacia senegal*) on C-reactive protein level among sickle cell anemia patients. BMC Res Notes. March,2020;13(1):162.
15. Sarkiyayi S, Abubakar MA. Effect of ethanol leaf extract of *Acacia nilotica* on phenyl hydrazine induced anemia in rats. Pharm Biol Sci. May,2018;13;1–10.
16. Afsar T, Razak S, Khan MR, Mawash S, Almajwal A, Shabir M, et al. Evaluation of antioxidant, anti-hemolytic and anticancer activity of various solvent extracts of *Acacia hydaspica* R. Parker aerial parts. BMC Complement Altern Med. July,2016;16(1);258.
17. Widowati R, Akati V, Arlym LT. Acacia honey consumption increases hemoglobin level of pregnant women with anemia. Purwestri YA, Sawitri WD, Nuringtyas TR, Amar A, Rohman MS, Minarsih H, et al., editors. BIO Web Conf. 2024;127:03002.
18. Kanbarkar N, Mishra S, Nandanwadkar S, Alegaon S. Assessment of anti-oxidant activity and quantification of epigallocatechin in *Acacia suma* heartwood by HPTLC-DPPH fingerprinting method. Chem Pap. September,2022;76(9);5865–78.
19. Saraf S, Ajazuddin. Evaluation of physicochemical and phytochemical properties of Safoof-E-Sana, a Unani polyherbal formulation. Phcog Res. September,2010;2(5);318.
20. Chinedum E, Kate E, Sonia C, Ironkwe A, Andrew I. Polyphenolic Composition and Antioxidant Activities of 6 New Turmeric (*Curcuma Longa L.*) Accessions. FNA. April,2015;7(1);22–7.
21. Kumar Agrawal K, Agrawal N, Jadon N, Gangwar K, Jain S, Sharma N, et al. Determination of Extractive Value, Phytochemical Constituents and In-Vitro Anti-Urolithiatic Activity of *Embelia ribes* Burm. F. and *Ipomea Hederacea* Jacq. *RJPT*. 2021;3566–70.
22. Khandelwal KR Sethi VK. Practical Pharmacognosy 13th edition. Pune, Nirali Prakashan 2019;25.1-25.9.p
23. Vaishnava S, Rangari VD. In-Vitro Anti-Sickling Activity of Selected Medicinal Plant to Explore Herbal Remedies for Sickle Cell Anemia. Int J Pharma Res Health Sci. 2019;7(1);2909–14.
24. Nouioura G, Lafdil FZ, Kandsi F, Mohammad Salamatullah A, Tiji S, Soulo N, et al. Unveiling the Antianemic Activity, Physicochemical Aspects, Antioxidant Properties, and Mineral Profile of *Petroselinum crispum L.* ACS Omega.June,2024;9(26);28155–66.

25. Dos Santos Magalhães IR, De Oliveira Soares A, Araújo LM, Da Costa PRC, De Araújo Roland I, Borrás MRL. Determination of Cu, Fe, Mn, and Zn in the Leaves and Tea of *Arrabidaea chica* (Humb. & Bompl.) Verl. Biol Trace Elem Res. December,2009;132(1–3);239–46.
26. Regupathi T, Chitra K. Physicochemical Analysis of Medicinal Herbs, *Eclipta Alba* (L.) Hassk and *Lippia Nodiflora* (Linn.). International Journal of Pharmaceutical and Phytopharmacological Research. 2014;4(4);249–51.
27. Kasthuri R, Ramesh B. Physicochemical and Fluorescence Analysis of leaves of *Alternanthera brasiliana* (L). Kuntze and *Alternanthera bettzickiana* (Regel) Voss. Int J Pharm Sci Rev Res.July,2018;51(1).
28. Asgary S, Naderi G, Askari N. Protective effect of flavonoids against red blood cell hemolysis by free radicals. Exp Clin Cardiol. 2005;10(2);88–90.
29. Lee HW, Kim H, Ryuk JA, Kil KJ, Ko BS. Hemopoietic effect of extracts from constituent herbal medicines of Samultang on phenylhydrazine-induced hemolytic anemia in rats. Int J Clin Exp Pathol. August,2014;7(9);6179–85.
30. Emilse LAM, Cecilia H, María TM, Eugenia MM, Alicia IB, Lazarte SS. Cryohemolysis, erythrocyte osmotic fragility, and supplementary hematimetric indices in the diagnosis of hereditary spherocytosis. Blood Res. March,2018;53(1);10.
31. Jeng SS, Chen YH. Association of Zinc with Anemia. Nutrients. November,2022;14(22);4918.
32. Abdelhaleim AF, Amer AY, Abdo Soliman JS. Association of Zinc Deficiency with Iron Deficiency Anemia and its Symptoms: Results from a Case-control Study. Cureus. January 2019;11(4);c20.
33. Kanbarkar N, Mishra S, Dodmani S, Kurangi B. Simultaneous estimation of epigallocatechin, fisetin, and quercetin in *Acacia suma* and its potential against postprandial hyperglycemia. IJAM. 2022;13(1);41–50.

*****