Research Article

Antioxidant Potential, Antidiabetic, and Anti-Inflammatory Activities of White Cowpea (*Vigna unguiculata* L.): An *in vitro* Study

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ABSTRACT

Aim/Background: Legumes are nutrient-dense with bioactive compounds offering protective effects against non-communicable diseases. The study aimed to screen phytochemicals, determine the total phenol and flavonoid contents and assess the *in vitro* antioxidant potential, and antidiabetic and anti-inflammatory activity of white cowpeas. **Materials and Methods:** Aqueous and ethanolic extracts of white cowpea were prepared. Total phenol content was estimated using the Folin-Ciocalteu colorimetric method and total flavonoid content was estimated using the aluminium chloride method. The antioxidant potential was assessed using DPPH assay, superoxide radical scavenging assay, phosphomolybdenum reduction assay and FRAP assay. The antidiabetic activity was evaluated using an alpha-amylase inhibition assay and the anti-inflammatory activity was evaluated using a human red blood cells membrane stabilization assay. **Results:** The total phenolic and total flavonoid content of white cowpea was two folds higher in the ethanol extract (343.67±1.19 µg/mg GAE and 60.87±0.33 µg/mg QE, respectively) than aqueous extract. Antioxidant analysis revealed that the ethanol extract exerted superior activity with an IC_{50} of 47.21±0.09 µg/mL against DPPH, 110.6±0.2 µg/mL against superoxide anions, 49.62±0.26 µg/mL against ferric while the aqueous extract had superior activity against phosphomolybdenum with an IC_{50} of 30.05±0.02 µg/mL. The anti-diabetic and anti-inflammatory analysis revealed superior activity of ethanol extract (30.88±0.51 µg/mL; 97.99±3.34 µg/mL, respectively) on par with the standard (acarbose and aspirin, respectively). The principal bioactive compounds present in white cowpea are ethyl α-d-glucopyranoside (43.24%), melezitose (18.25%) and 3-O-Methyl-d-glucose (11.19%). **Conclusion:** White cowpeas are rich in bioactive compounds with excellent antioxidant potential, anti-diabetic and anti-inflammatory activity.

Keywords: Antioxidant, Antidiabetic, Anti-Inflammatory, White Cowpea.

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INTRODUCTION

Legumes are members of the *Fabaceae* or *Leguminosae* family that bear edible seeds in pods. They are considered the second most important food source after cereals.[1-3] Legumes hold high economic and environmental importance as cultivation of legumes dramatically reduces the use of synthetic fertilizers by establishing symbiotic relationship with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi, thus reducing the release of a major greenhouse gas NO_2.[4-6]

Legumes commonly used for human consumption include soybeans, common beans, chickpeas, cowpeas, dry peas, lentils, lupins, green peas, pigeon peas, groundnuts and peanuts.[1,4] They are a sustainable source of proteins, dietary fibre, starch, vitamins, and minerals containing significant amounts of phytochemicals that confer beneficial effects on human health.[7] Several studies have revealed that consumption of legumes has

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a protective effect against non-communicable diseases.[7,8] In a recently published study, raw and cooked black turtle beans (*Phaseolus vulgaris* L.) possessing diverse profiles of bioactive compounds were found to exhibit excellent *in vitro* anticancer activity against human colorectal adenocarcinoma (HT-29) cells and human breast adenocarcinoma (MCF-7) cells.[9] However, phenolic compounds that confer these benefits majorly tend to vary with different legumes due to seed coat color.[10] Hence, the study aimed to screen phytochemicals, estimate the total phenol and flavonoid contents, assess the *in vitro* antioxidant, antidiabetic, anti-inflammatory activity and identify potential bioactive compounds present in white cowpea.

MATERIALS AND METHODS

Raw Materials

White cowpea (*Vigna unguiculata* L.) was procured from a local vendor in Chennai, Tamil Nadu, India. White cowpea was checked for any stones, chaff and plant parts, washed with distilled water and dried under sunlight. The legume seeds were ground in a hammer mill and sieved through 0.2-mm screen. The finely powdered legumes were stored in labelled air-tight containers for analysis.

Proximate Analysis

The moisture, carbohydrate, protein, fat, crude fibre and ash were determined according to AOAC.[11] Energy (kcal) was calculated.

Preparation of Extracts

Aqueous and ethanol extract of white cowpea were prepared for the screening of phytochemicals, estimation of total phenol and flavonoid, *in vitro* antidiabetic and anti-inflammatory activity. The aqueous extract was prepared using the hot water extraction method. In a 250 mL sterile conical flask, five gram of pulverized white cowpea was mixed with 50 mL distilled water before sealing it with a cotton plug. The mixture was autoclaved for 10 min and filtered using filter paper. The resultant supernatant was stored at 5℃ in air-tight glass bottles. The ethanol extract was prepared using the maceration method. Five grams of pulverized white cowpea was soaked in 50 mL ethanol for 72 hr. The resultant substance was filtered and stored at 5℃ in air-tight glass bottles.

Screening of Phytochemicals

The phytochemicals present in the aqueous and ethanol extract of white cowpea were screened using standard methods.[12] Test for alkaloids, terpenoids, steroids, phenols, flavonoids, tannins and saponins were carried out to check for presence/absence.

Estimation of Total Phenolic Content

The total phenolic content of white cowpea was determined using Folin-ciocalteau method.[13] Aqueous and ethanol extract (0.5 mL each) were mixed with 2.5 mL of Folin-ciocalteau reagent (10%) and 2.5 mL of sodium bicarbonate (7.5%) was added after five min. This mixture was incubated in the dark for 45 min at room temperature and the absorbance was measured using UV-Spectrophotometer at 765 nm. Gallic acid was used as the standard and the results were presented as µg/mg of GAE.

Determination of Total Flavonoid Content

The total flavonoid content of white cowpea was determined using the aluminium chloride method.[14] Based on this method, the aqueous and ethanol extract (1.0 mL each) were mixed with 4 mL of distilled water, 0.30 mL of $NaNO_2$ solution (10%), 0.30 mL $AlCl_3$ solution (10%) and 2.0 mL of NaOH solution (1%). The mixture was thoroughly mixed and absorbance was measured using UV-Spectrophotometer at 510 nm. Quercetin was used as the standard and the results were expressed as µg/mg of QE.

Antioxidant Assays

In vitro antioxidant potential of white cowpea was assessed using free radical scavenging assays namely DPPH free radical scavenging assay and superoxide anion radical scavenging assay and by reduction assays namely phosphomolybdenum reduction assay and ferric reducing antioxidant power assay.

DPPH Assay

DPPH free radical scavenging assay was carried out with slight modification.[15] Aqueous and ethanol extract of white cowpeas at different concentrations (20,40,60,80,100 and 120µg/mL each) reconstituted with methanol were added to 5mL of DPPH (0.1mM) prepared in methanol. As a control, 5 mm of DPPH solution in methanol without antioxidants was utilized. The test tubes were vigorously shaken and incubated at 25ºC for 30 min. A spectrophotometer was used to measure the absorbance at 517 nm. The percentage of radical scavenging activity was calculated using the following formula:

$$\% = \left[\frac{\text{Absorbance in Control} - \text{Absorbance in Sample}}{\text{Absorbance in Control}}\right] \times 100$$

Superoxide Anion Radical Scavenging Assay

In vitro antioxidant potential of white cowpea was determined using a superoxide anion radical scavenging assay.[16] Aqueous and ethanol extract of white cowpeas at different concentrations (20, 40, 60, 80, 100, and 120 µg/mL each) were taken and 50 mM phosphate buffer (pH 7.8), 1.5mM of riboflavin, 12 mM of EDTA, and 50 mM mg NBT were added. The resulting mixes were held for 10 min under a UV lamp. Using a UV-Spectrophotometer, the extracts' absorbance was assessed following the incubation time at 580 nm. The percentage of radical scavenging activity was calculated using the following formula:

$$\% = \left[\frac{\text{Absorbance in Control} - \text{Absorbance in Sample}}{\text{Absorbance in Control}}\right] \times 100$$

Phosphomolybdenum Reduction Assay

Phosphomolybdenum reduction assay was carried out.[17] Aqueous and ethanol extract of white cowpea (20, 40, 60, 80, 100, 120 µg/mL each) were mixed with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 M sodium phosphate, and 4 M ammonium molybdate). Methanol (0.1 mL) was used as the sample substitute in the blank. Following a 90 min incubation period at 95ºC in a bath of boiling water, the tubes were sealed with aluminium foil sheets. The absorbance was noted at 695 nm using a UV-Spectrophotometer. The percentage of phosphomolybdenum reduction was calculated using the following formula:

$$\% = \left[\frac{\text{Absorbance in Sample} - \text{Absorbance in Control}}{\text{Absorbance in Sample}}\right] \times 100$$

FRAP Assay

Ferric Reducing Antioxidant Power (FRAP) assay was carried out with slight modification.[15] Aqueous and ethanol extract of white cowpeas were taken at 20,40,60,80,100,120 µg/mL concentrations (each) and were mixed with 2.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of potassium ferricyanide (1%) solution. Following 20 min of incubation at 50ºC, 10% tricholoroacetic acid was added to the mixture and thoroughly mixed. The mixture was added with 1 mL of freshly prepared ferric chloride (0.1%), which was then incubated at 35ºC for 10 min. The absorbance at 700 nm was then measured using a UV-Spectrophotometer. The percentage of FRAP assay was calculated using the following formula:

$$\% = \left[\frac{\text{Absorbance in Sample} - \text{Absorbance in Control}}{\text{Absorbance in Sample}}\right] \times 100$$

Antidiabetic Assay

In vitro antidiabetic activity of white cowpea was determined using the α-amylase inhibition assay (starch-iodine test).[18] Aqueous and ethanol extract of white cowpea were taken at concentrations of 20, 40, 60, 80, 100 and 120 µg/mL (each) and 500 µL of 0.2M sodium phosphate buffer (pH 6.9 containing 6 mM sodium chloride) was added. The resultant mixture was incubated for 10 min at room temperature after the addition of α-amylase solution (10 µL). Soluble starch (1%, w/v) of 500 µL was added and incubated at 37ºc for 10 min. The addition of dilute HCl (100 µL) and iodine solution (200 µL) was done to stop the enzymatic reaction. Acarbose was used as the standard reference. The absorbance was noted using a UV-Spectrophotometer at 620 nm. The percentage of inhibition was calculated as follows:

$$\% = \left[\frac{\text{Absorbance in Control} - \text{Absorbance in Sample}}{\text{Absorbance in Control}}\right] \times 100$$

Anti-inflammatory Assay

In vitro anti-inflammatory activity of white cowpea was estimated using Human RBC (HRBC) membrane stabilization method.[19] HRBC suspension was prepared with two mL of blood obtained from a healthy volunteer who had not taken NSAIDs two weeks prior to the experiment. The collected blood was centrifuged at 3000 rpm for 5 min and the separated packed cells were washed with normal saline solution (0.9% NaCl) and reconstituted as a 10% (v/v) suspension with isotonic buffer solution (10 mM sodium phosphate buffer pH 7.4). Aqueous and ethanol extract of white cowpea were taken at 20, 40, 60, 80, 100 and 120 µg/mL (each) and were mixed with 2.95 mL of phosphate buffer (pH 7.4) and 0.5 mL of HRBC suspension. The resultant mixtures were incubated at 54ºC for 20 min and centrifuged at 2500 rpm for 3 min. The supernatant liquid was decanted and the absorbance was measured at 540 nm using a UV-Spectrophotometer. Aspirin was used as the

standard reference. The percentage of hemolysis using the following formula:

$$\text{Prevention of lysis} = 100 - \left[\frac{\text{Absorbance in sample}}{\text{Absorbance in control}}\right] \times 100$$

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

GC-MS analysis was used to identify bioactive compounds present in white cowpea.[9] The bioactive compounds were identified using the library spectra database of the National Institute of Standard and Technology (NIST).

Statistical Analysis

The analysis was performed in triplicates. The difference in phytochemical composition between the extracts was analysed using a student *t*-test. The half maximal inhibition potential (IC_{50}) was calculated from the dose-response non-linear regression curve fit for *in vitro* antioxidant, antidiabetic and anti-inflammatory assay by plotting percentage inhibition versus concentrations. The difference in IC_{50} between extracts was analysed using one-way ANOVA. The results were considered significant at p value of <0.05 and were reported as mean±standard deviation. GraphPad Prism Software, Version 9 was used for statistical analysis.

RESULTS

Proximate Composition

From Table 1, it can be observed that white cowpea contains moisture of 11.45±0.64%, the energy of 348.36±0.75 kcal/100g, carbohydrate of 63.99±0.58 g/100g, protein of 19.05±0.78 g/100g, fat of 1.85±0.86 g/100g, the crude fiber of 3.40±0.50 g/100g and ash of 3.55±0.43 g/100g.

Phytochemicals

Screening of phytochemicals revealed the presence of alkaloids, terpenoids, steroids, phenols, flavonoids, tannins and saponins in both aqueous and ethanol extract of white cowpeas.

Table 1: Proximate composition of white cowpea (*Vigna unguiculata* L.).

Constituents	Composition
Energy (kcal/100g)	348.36±0.75
Carbohydrate (g/100g)	63.99±0.58
Protein (g/100g)	19.05±0.78
Fat (g/100g)	1.85±0.86
Crude Fiber (g/100g)	3.40±0.50
Ash	3.55±0.43

Table 2: Total phenol and flavonoid present in aqueous and ethanol extract of white cowpea (*Vigna unguiculata* L.).

Phytochemicals	Aqueous Extract	Ethanol Extract
Total Phenol (µg/mg GAE)	117.2±0.30	343.67±1.19**
Total Flavonoid (µg/mg QE)	30.03±0.21	60.87±0.33**

Note: GAE-Gallic Acid Equivalent; QE-Quercetin Equivalent, ** p value <0.01

Total Phenol and Total Flavonoid Content

As presented in Table 2, both aqueous and ethanol extract of white cowpea revealed the presence of total phenols and total flavonoids, with ethanol extract presenting with highest contents. This demonstrates the efficacy of ethanol to extract phenolic and flavonoid compounds better than aqueous solvent.

Antioxidant Potential

Figure 1 illustrates dose dependent *in vitro* antioxidant potential of aqueous and ethanol extract of white cowpea at concentrations ranging between 20-120 µg/mL. Ethanol extract of white cowpea was found to exhibit increased free radical scavenging activity dose dependently by degrading DPPH radical, superoxide anions and by reducing molybdenum and ferric dose dependently when compared to the aqueous extract.

Half-maximal inhibition (IC_{50}) is defined as the concentration required to inhibit a compound where the lower the value greater the potential. The IC_{50} values of the aqueous and ethanol extract of white cowpea against DPPH, superoxide anion radicals, phosphomolybdenum and ferric are presented in Table 3. It can be observed that ethanolic extract had a significantly lower IC_{50} value except for phosphomolybdenum reducing power ($p<0.01$). However, the antioxidant potential of both aqueous and ethanol extract were weaker than the standard ascorbic acid ($p<0.01$).

Anti-diabetic Activity

The ability of aqueous and ethanol extract of white cowpea to inhibit **α-amylase enzyme** is illustrated in Figure 2. The inhibition of enzyme by the legumes was found to be dose dependent at increasing concentrations (20-120 µg/mL) for aqueous and ethanol extract. The ethanol extract of white cowpea had increased inhibitory activity than the aqueous extract. The half maximal inhibition potential (IC_{50}) of aqueous extract was 52.73±3.75 µg/mL, ethanol extract was 30.88±0.51 µg/mL and acarbose (standard) was 28.99±0.17 µg/mL as

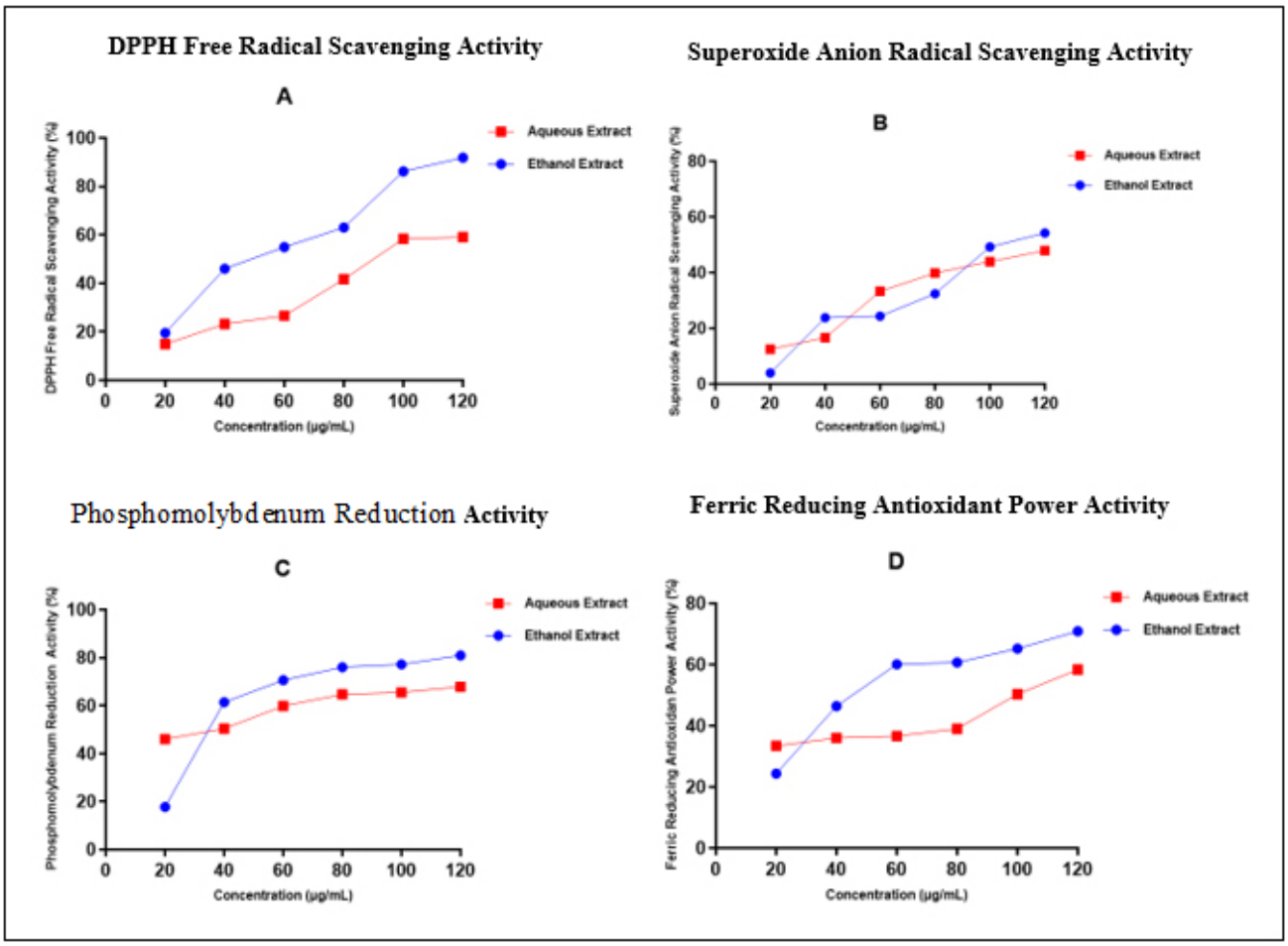


Figure 1: *In vitro* antioxidant potential of aqueous extract of white cowpea (*Vigna unguiculata* L.).

Table 3: IC_{50} values of white cowpea (*Vigna unguiculata* L.) for *in vitro* antioxidant, antidiabetic and anti-inflammatory activity.

Sample	IC_{50} Values					
	Antioxidant Activity (µg/mL)				Antidiabetic Activity: Alpha-amylase inhibition assay (µg/mL)	Anti-inflammatory Activity:Human RBC membrane stabilization assay (µg/mL)
	DPPH free radical scavenging assay	Superoxide anion radical scavenging assay	Phosphomolybdenum Reduction assay	FRAP assay		
Aqueous Extract	93.93±0.20[a]	122.3±0.71[a]	30.05±0.02[a]	110±1.27[a]	52.73±3.75[a]	759.76±12.61[a]
Ethanol Extract	47.21±0.09[b]	110.6±0.2[b]	38.12±0.30[b]	49.62±0.26[b]	30.88±0.51[b]	97.99±3.34[b]
Standard	7.06±0.07[c]	6.41±0.02[c]	1.21±0.10[c]	1.72±0.18[c]	28.94±2.15[b]	108.04±0.45[b]

Note: Different superscripts within column indicate significant difference (p<0.01).

presented in Table 3. It can be inferred that the IC_{50} potential of the aqueous extract of white cowpea is significantly weaker than acarbose (p<0.01) while that of the ethanol extract was marginally closer to acarbose with no significant difference.

Anti-inflammatory Activity

Figure 3 illustrates dose dependent anti-inflammatory activity of white cowpea at concentrations of 20-120 µg/mL. The IC_{50} values of aqueous extract of white cowpea was 759.76±12.61 µg/mL, ethanol extract was 97.99±3.34 µg/mL and aspirin (standard) was 108.04±0.45 µg/mL (Table 3). These indicate that the HRBC membrane stabilization potential of aqueous extract of white cowpea was almost seven fold lower than aspirin (p<0.01), whereas the potential of ethanol extract was higher than the standard aspirin. However, the difference between the IC_{50} of ethanol extract and aspirin was not statistically significant.

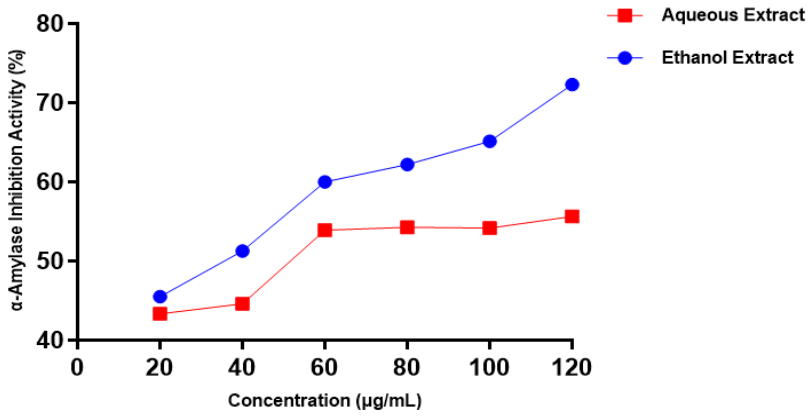


Figure 2: *In vitro* antidiabetic activity of white cowpea (*Vigna unguiculata* L.).

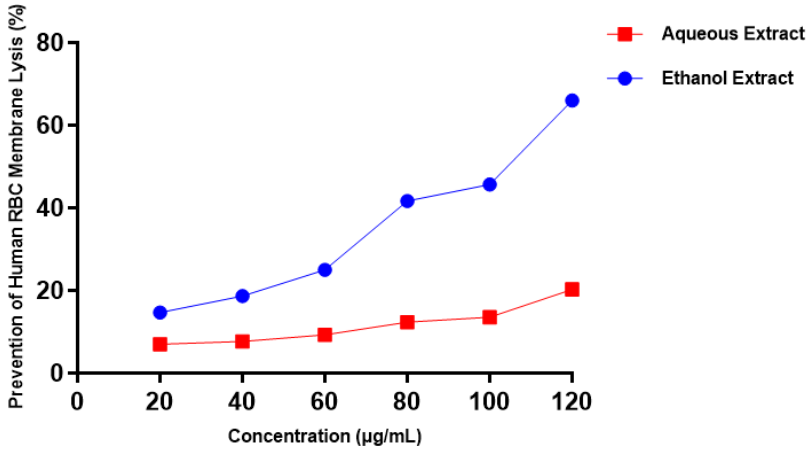
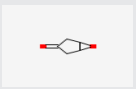
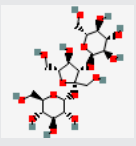
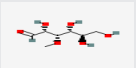
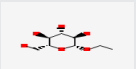


Figure 3: *In vitro* anti-inflammatory activity of white cowpea (*Vigna unguiculata* L.).

Bioactive Compounds

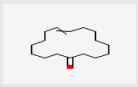
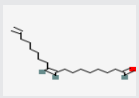
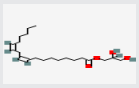
Table 4 presents the bioactive compounds present in white cowpea. The principal bioactive compounds present are ethyl α-d-glucopyranoside (43.24%), melezitose (18.25%) and 3-O-Methyl-d-glucose (11.19%).

Table 4: Bioactive compounds present in the ethanol extract of white cowpea (Vigna unguiculata).

Sl. No.	Compound Name	Structure	RT	Peak Area (%)
1.	6-Oxa- bicyclo[3.1.0]hexan-3- one		3.206	3.01
2.	Melezitose		13.87	18.25
3.	3-O-Methyl-d-glucose		17.33	11.19
4.	Ethyl α-d-glucopyranoside		18.321	43.24

Continued...

Table 4: Cont'd.

Sl. No.	Compound Name	Structure	RT	Peak Area (%)
6.	n-Hexadecanoic acid		26.516	2.82
7.	Hexadecanoic acid, ethyl ester		27.417	4.19
8.	1,E-11,Z-13-Octadecatriene		30.580	1.79
9.	2H-Pyran,2-(2-heptadecynyloxy)tetrahydro-		30.717	1.53
10.	9€,11(E)-Conjugated linoleic acid, ethyl ester		31.230	1.99
11.	8-Cyclohexadecen-1-one		31.374	1.60
12.	9,17-Octadecadienal, (Z)-		34.39	0.55
13.	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester		37.512	2.61
14.	9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester		40.281	4.13

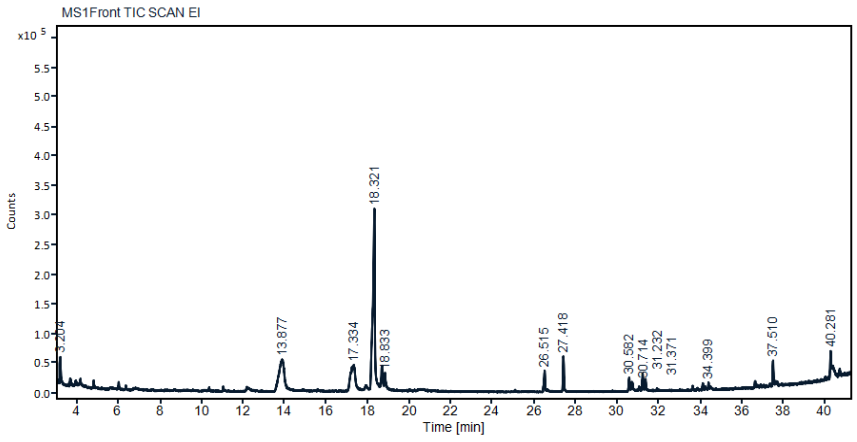


Figure 4: Presence of bioactive compounds in white cowp–a - GC-MS analysis.

DISCUSSION

The present study elucidated the health promoting effect of white cowpeas, unveiling its potential as a functional food. With respect to the proximate composition, the moisture and carbohydrate content of white cowpeas were higher while the protein content was lower when compared to other studies.[20,21] The fat content was similar to Sasanam *et al*.[20] and much lower than the values reported by Alfa *et al*.[21] The crude fiber of white cowpea was marginally lower than the results of Alfa *et al*.[21] while the ash content was marginally higher. Variations in the proximate composition of white cowpeas across studies could be due to the difference in species, geographical location, climate and soil conditions.

Qualitative analysis of phytochemicals in the aqueous and ethanol extract of white cowpeas revealed the presence of alkaloids, terpenoids, steroids, phenols, flavonoids,

tannins and saponins. This is in agreement with other studies.[22-24] Quantitative estimation of total phenols and total flavonoids in white cowpea revealed reported that the ethanol extract had higher amounts than the aqueous extract. Adjei-Fremah *et al*.[25] confirmed the presence of phenolic content in several varieties of cowpeas. Phenols and flavonoids are responsible for various beneficial biological processes, including radical scavenging, enzyme inhibition and exerts antioxidant, anti-inflammatory, anti-hypertensive, anti-lipidemic and antimicrobial activity.[26] Jayathilake *et al*.[27] reported that the phenolic content of the entire cowpeas is majorly composed of free phenolics, with the seed coat having 5 to 10 times greater phenolic concentration and around ten times the flavonoids content.

Antioxidants are gaining increasing importance owing to their health benefits. In the present study, white cowpea was found to exert excellent antioxidant activity. In comparison, the ethanol extract of white cowpea exhibited greater *in vitro* antioxidant activity except for phosphomolybdenum reducing assay where the aqueous extract had greater potential. This beneficial effect of white cowpea is attributed to its rich phenol and flavonoid profile as they are said to be the key players in phytochemical-mediated free-radical scavenging.[28] Xu and Chang[29] reported a strong positive association was found between phenolic content and antioxidant activities. Sombie *et al*.,[30] reported that different varieties of cowpea exhibited DPPH scavenging potential of seeds but the white variety had the highest potential. Whereas, for the ferric reducing power, cowpeas with coloured seed coats had the highest potential.

With respect to the antidiabetic potential, results demonstrated excellent inhibitory activity of white cowpea against α-amylase enzyme. The ethanol extract exhibited the highest α-amylase inhibitory activity when compared with aqueous extract. These results highlight α-amylase inhibitory activity as one of the mechanisms by which white cowpeas can potentially lower blood glucose levels. Since the digestion of carbohydrates is facilitated by salivary and pancreatic α-amylase enzymes, inhibition of this enzyme is one of the possible ways to treat high postprandial blood glucose levels.[31] The possible mechanism behind this effect can be attributed to phenolic compounds that are reported to inhibit starch digestive enzymes by non-covalent interactions in addition to direct interactions.[32,33] Sreerama *et al*.,[34] in his study revealed that defatted cowpea inhibited α-amylase enzyme which is stronger than the results of the present study. The difference in the inhibition capacity is due to the variation in cultivar, colour of the seed coat, growing and processing conditions.

Heat-induced HRBC membrane lysis reflects the anti-inflammatory potential of an extract because human RBC membranes are analogs to lysosomal membrane components.[35] During inflammation, rupture of the lysosomes membrane results in the release of hydrolytic enzymes of lysosomes that trigger inflammatory reactions. Hence, stabilization of HRBC membrane is considered an *in vitro* marker of a compound's anti-inflammatory potential.[36] Results of the present study indicated that the ethanolic extract of white cowpea exhibited the most significant HRBC membrane lysis potential when compared to the aqueous extract. This can be attributed primarily to their functional pigments i.e phenolic compounds.[37,38] The phenolic compounds scavenge free radicals and reduce lipid peroxidation thereby leading to stabilization of the cell membranes.[39] However, other compounds namely saponins, proteins and peptides present in legume seeds are also known to exert anti-inflammatory effects through other mechanisms.[37,38]

In the present study, GC-MS analysis revealed the presence of bioactive compounds in white cowpea primarily comprising of ethyl α-d-glucopyranoside, melezitose and 3-O-Methyl-d-glucose. Ethyl α-d-glucopyranoside, is a glycoside which has excellent radical scavenging activity.[40] Based on a molecular docking study by Rashid *et al*.,[41] ethyl α-d-glucopyranoside was found to exert the highest binding affinity against alpha-amylase enzyme thereby confirming the antidiabetic effect. Melezitose is a type of oligosaccharide and in a study by Rodríguez-Flores *et al*.,[42] it was found that melezitose alongside of phenol and flavonoids showed linear relationship with total antioxidant effect. 3-O-Methyl-d-glucose is a sugar moiety and 9,12-Octadecadienoic acid (Z,Z)-, 2,3-dihydroxypropyl ester is an unsaturated fatty acid.[43,44] Identification of hydrocinnamic acid, o-[(1,2,3,4-tetrahydro-2-naphthyl) methyl] is significant because cinnamic acid derivatives are linked to the prevention of oxidative stress-related diseases.[45] Thus, bioactive compounds present in white cowpeas work synergistically to confer antioxidant potential, antidiabetic and anti-inflammatory activity.

CONCLUSION

Results show that the aqueous and ethanol extract of white cowpeas are rich in phenol and flavonoid with excellent antioxidant potential, anti-diabetic and anti-inflammatory activity. The abundance of bioactive

compounds may be responsible for its beneficial effect, thereby rendering it a good alternative for the commonly consumed legumes as they can potentially reduce oxidative stress, elevated blood glucose levels and inflammation thereby lowering the risk of non-communicable diseases.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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ABBREVIATIONS

AlC_3: Aluminium Chloride; **ANOVA:** Analysis of Variance; **AOAC:** Association of Official Analytical Collaboration; **DPPH:** 2,2-diphenylpicrylhydrazyl; **EDTA:** Ethylenediaminetetraacetic Acid; **FRAP:** Ferric Reducing Antioxidant Power; **GAE:** Gallic Acid Equivalent; **GC-MS:** Gas Chromatography Mass Spectrometry; **HRBC:** Human Red Blood Cell; **HCl:** Hydrochloric acid; **IC_{50}:** Inhibition Concentration at 50%; **Kcal:** Kilocalorie; **$NaNO_2$:** Sodium Nitrite; **NBT:** Nitroblue Tetrazolium Test; **NIST:** National Institute Standard and Technology; **No_2:** Nitrogen dioxide; **NSAIDs:** Non-Steroidal Anti-Inflammatory Drugs; **QE:** Quercetin Equivalent; **UV:** Ultra Violet.

SUMMARY

The present study assessed the *in vitro* antioxidant, antidiabetic and anti-inflammatory activities of white cowpeas. Aqueous and ethanol extracts were prepared for the analysis. Qualitative screening of phytochemicals revealed the presence of alkaloids, terpenoids, steroids, phenols, flavonoids, tannins and saponins in both extracts of white cowpea. Quantitative estimation of the total phenols and flavonoids revealed higher amounts in ethanol extract of white cowpea. With respect to *in vitro* assays, results demonstrated noteworthy antioxidant, antidiabetic and anti-inflammatory activity suggesting its potential role in the prevention and management of non-communicable diseases. GC-MS analysis provided valuable insights by identifying the principal bioactive compounds present in white cowpea that are responsible for their beneficial effects. This *in vitro* study on white cowpea's health promoting benefits provides a scientific basis and paves the way for future studies to further explore their potential in disease prevention and management.

REFERENCES

1. Temba MC, Njobeh PB, Adebo OA, Olugbile AO, Kayitesi E. The role of compositing cereals with legumes to alleviate protein energy malnutrition in Africa. Int J Food Sci Technol. 2016;51(3):543-54. doi: 10.1111/ijfs.13035.
2. Çakir Ö, Uçarli C, Tarhan Ç, Pekmez M, Turgut-Kara N. Nutritional and health benefits of legumes and their distinctive genomic properties. Food Sci Technol. 2019;39(1):1-12. doi: 10.1590/fst.42117.
3. Gebrelibanos M, Tesfaye D, Raghavendra Y, Sintayeyu B. Nutritional and health implications of legumes. Int J Pharm Sci Res. 2013;4(4):1269.
4. Kamboj R, Nanda V. Proximate composition, nutritional profile and health benefits of legumes-A review. Legume Res Int J. 2018;41(Of). doi: 10.18805/LR-3748.
5. Castro-Guerrero NA, Isidra-Arellano MC, Mendoza-Cozatl DG, Valdés-López O. Common bean: a legume model on the rise for unraveling responses and adaptations to iron, zinc, and phosphate deficiencies. Front Plant Sci. 2016;7:600. doi: 10.3389/fpls.2016.00600, PMID 27200068.
6. Goyal RK, Mattoo AK, Schmidt MA. Rhizobial–host interactions and symbiotic nitrogen fixation in legume crops toward agriculture sustainability. Front Microbiol. 2021;12:669404. doi: 10.3389/fmicb.2021.669404, PMID 34177848.
7. Silva LR, Bento C, Gonçalves AC, Flores-Félix JD, Ramírez-Bahena MH, Peix A, *et al*. Legume bioactive compounds: influence of rhizobial inoculation. AIMS Microbiol. 2017;3(2):267-78. doi: 10.3934/microbiol.2017.2.267, PMID 31294160.
8. Carbas B, Machado N, Pathania S, Brites C, Rosa EA, Barros AI. Potential of legumes: nutritional value, bioactive properties, innovative food products, and application of eco-friendly tools for their assessment. Food Rev Int. 2021:1-29.
9. Priyadarshini RD, Beatrice DA. Anticancer activity of black turtle bean against breast and colorectal adenocarcinoma: A pre-clinical study. Asian J Biol Life Sci. 2022;11(1):193-9. doi: 10.5530/ajbls.2022.11.27.
10. Alcázar-Valle M, Lugo-Cervantes E, Mojica L, Morales-Hernández N, Reyes-Ramírez H, Enríquez-Vara JN, *et al*. Bioactive compounds, antioxidant activity, and antinutritional content of legumes: a comparison between four Phaseolus species. Molecules. 2020;25(15):3528. doi: 10.3390/molecules25153528, PMID 32752304.
11. AOAC. Official methods of analysis of the Association of Analytical Chemists International. Official methods. Gaithersburg, MD; 2005.
12. Sachin C, Arvind N, Vinesh D. The study of *in vitro* antimicrobial activity and phytochemical analysis of some medicinal plants in Chamoli Garhwal Region. Pharmacogn J. 2010;2(12):481-5. doi: 10.1016/S0975-3575(10)80035-5.
13. Niroula A, Khatri S, Khadka D, Timilsina R. Total phenolic contents and antioxidant activity profile of selected cereal sprouts and grasses. Int J Food Prop. 2019;22(1):427-37. doi: 10.1080/10942912.2019.1588297.
14. Katırcı N, Işık N, Güpür Ç, Guler HO, Gursoy O, Yilmaz Y. Differences in antioxidant activity, total phenolic and flavonoid contents of commercial and homemade tomato pastes. J Saudi Soc Agric Sci. 2020;19(4):249-54. doi: 10.1016/j.jssas.2018.11.003.
15. Sasikumar JM, Erba O, Egigu MC. *In vitro* antioxidant activity and polyphenolic content of commonly used spices from Ethiopia. Heliyon. 2020;6(9):e05027. doi: 10.1016/j.heliyon.2020.e05027, PMID 32995654.
16. Zemmouri H, Ammar S, Boumendjel A, Messarah M, El Feki A, Bouaziz M. Chemical composition and antioxidant activity of *Borago officinalis L.* leaf

extract growing in Algeria. Arab J Chem. 2019;12(8):1954-63. doi: 10.1016/j.arabjc.2014.11.059.
17. Muritala HF, Akolade JO, Akande SA, Abdulazeez AT, Aladodo RA, Bello AB. Antioxidant and alpha-amylase inhibitory potentials of *Cocos nucifera* husk. Food Sci Nutr. 2018;6(6):1676-83. doi: 10.1002/fsn3.741, PMID 30258612.
18. Nasir A, Khan M, Rehman Z, Khalil AAK, Farman S, Begum N, *et al*. Evaluation of alpha-amylase inhibitory, antioxidant, and antimicrobial potential and phytochemical contents of *Polygonum hydropiper* L. Plants (Basel). 2020;9(7):1-13. doi: 10.3390/plants9070852, PMID 32640649.
19. Gunathilake KDPP, Ranaweera KKDS, Rupasinghe HPV. *In vitro* anti-inflammatory properties of selected green leafy vegetables. Biomedicines. 2018;6(4):1-10. doi: 10.3390/biomedicines6040107, PMID 30463216.
20. Sasanam S, Paseephol T, Moongngarm A. Comparison of proximate compositions, resistant starch content, and pasting properties of different colored cowpeas (*Vigna unguiculata*) and red kidney bean (*Phaseolus vulgaris*). Int J Nutr Food Eng. 2011;5(9):553-7.
21. Alfa AA, Tijani KB, Omotoso OD, Junaidu Y, Sezor AA. Nutritional values and medicinal health aspects of brown, brown-black and white cowpea (*Vigna unguiculata* L. Walp.) grown in Okene, Kogi state, Nigeria. Asian J Adv Res. 2020;14(4):114-24. doi: 10.9734/ajarr/2020/v14i430348.
22. Sani UM. Phytochemical screening and antifeedant activity of the seed extracts of *Parkia biglobosa* against cowpea vean (*Vigna unguiculata*) storage pest (*Callosobruchus maculatus*). Int J Innov Sci Eng Technol. 2014;3(9):15991-5.
23. Londonkar RL, Awanti BS. Pharmacognostic and phytochemical screening of cowpea seeds (*Vigna unguiculata*). Int J Phytomed. 2014;6(4):510-4.
24. Allah NSK, Eltayeb IM, Hamad AEH. Phytochemical screening and hypolipidemic activity of extracts from seeds and leaves of *Vigna unguiculata* growing in Sudan. J Pharmacogn Phytochem. 2017;6(3):488-91.
25. Adjei-Fremah S, Jackai LE, Worku M. Analysis of phenolic content and antioxidant properties of selected cowpea varieties tested in bovine peripheral blood. Am J Anim Vet Sci. 2015;10(4):235-45. doi: 10.3844/ajavsp.2015.235.245.
26. Adekola MB, Areola JO, Fagbohun OF, Asaolu FT, Ogundepo GE, Fajobi AO, *et al*. *In vitro* antioxidant and anti-inflammatory activities of ethanol stem-bark extract of *Blighia sapida* KD Koenig. J Pharm Anal. 2022;12(2):350-4. doi: 10.1016/j.jpha.2021.04.002, PMID 35582399.
27. Jayathilake C, Visvanathan R, Deen A, Bangamuwage R, Jayawardana BC, Nammi S, *et al*. Cowpea: an overview on its nutritional facts and health benefits. J Sci Food Agric. 2018;98(13):4793-806. doi: 10.1002/jsfa.9074, PMID 29656381.
28. Prasathkumar M, Raja K, Vasanth K, Khusro A, Sadhasivam S, Sahibzada MUK, *et al*. Phytochemical screening and *in vitro* antibacterial, antioxidant, anti-inflammatory, anti-diabetic, and wound healing attributes of *Senna auriculata* (L.) Roxb. leaves. Arab J Chem. 2021;14(9):103345. doi: 10.1016/j.arabjc.2021.103345.
29. Xu B, Chang SKC. Comparative study on antiproliferation properties and cellular antioxidant activities of commonly consumed food legumes against nine human cancer cell lines. Food Chem. 2012;134(3):1287-96. doi: 10.1016/j.foodchem.2012.02.212, PMID 25005945.
30. Sombié PA, Compaoré M, Coulibaly AY, Ouédraogo JT, Tignégré JB, Kiendrébéogo M. Antioxidant and phytochemical studies of 31 cowpea (*Vigna unguiculata* (L. Walp.)) genotypes from Burkina Faso. Foods. 2018;7(9):143.
31. García-Díaz YD, Aquino-Bolaños EN, Chávez-Servia JL, Vera-Guzmán AM, Carrillo-Rodríguez JC. Bioactive compounds and antioxidant activity in the common bean are influenced by cropping season and genotype. Chil J Agric Res. 2018;78(2):255-65. doi: 10.4067/S0718-58392018000200255.
32. Mechchate H, Es-Safi I, Conte R, Hano C, Amaghnouje A, Jawhari FZ, *et al*. *In vivo* and *in vitro* antidiabetic and anti-inflammatory properties of Flax (*Linum usitatissimum* L.) seed polyphenols. Nutrients. 2021;13(8):2759. doi: 10.3390/nu13082759, PMID 34444919.
33. Martinez-Gonzalez AI, Díaz-Sánchez ÁG, de la Rosa LA, Vargas-Requena CL, Bustos-Jaimes I, Alvarez-Parrilla AE. Polyphenolic compounds and digestive enzymes: *in vitro* non-covalent interactions. Molecules. 2017;22(4):669. doi: 10.3390/molecules22040669, PMID 28441731.
34. Sreerama YN, Sashikala VB, Pratape VM. Phenolic compounds in cowpea and horse gram flours in comparison to chickpea flour: evaluation of their antioxidant and enzyme inhibitory properties associated with hyperglycemia and hypertension. Food Chem. 2012;133(1):156-62. doi: 10.1016/j.foodchem.2012.01.011.
35. Aleixandre A, Gil JV, Sineiro J, Rosell CM. Understanding phenolic acids inhibition of α-amylase and α-glucosidase and influence of reaction conditions. Food Chem. 2022;372:131231. doi: 10.1016/j.foodchem.2021.131231, PMID 34624776.
36. Chowdhury A, Azam S, Jainul MA, Faruq KO, Islam A. Antibacterial activities and *in vitro* anti-inflammatory (membrane stability) properties of methanolic extracts of *Gardenia coronaria* leaves. Int J Microbiol. 2014;2014:410935. doi: 10.1155/2014/410935, PMID 24695677.
37. Ruiz-Ruiz JC, Matus-Basto AJ, Acereto-Escoffié P, Segura-Campos MR. Antioxidant and anti-inflammatory activities of phenolic compounds isolated from *Melipona beecheii* honey. Food Agric Immunol. 2017;28(6):1424-37. doi: 10.1080/09540105.2017.1347148.
38. Qubra K, Singh B. Study of *in vitro* anti-inflammatory property of *Dendrocnide sinuata* (*Blume*) *Chew and Chenopodium ambrosioides* (L.): ethnomedicinal plants from Assam. In: Ghannam WM, editor. Trends in Pharmaceutical Research and Development. Vol. 2. India: Book Publisher International; 2020. p. 138-44.
39. Zhu F, Du B, Xu B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: a review. Crit Rev Food Sci Nutr. 2018;58(8):1260-70. doi: 10.1080/10408398.2016.1251390, PMID 28605204.
40. Trincone A, Pagnotta E, Tramice A. Enzymatic routes for the production of mono- and di-glucosylated derivatives of hydroxytyrosol. Bioresour Technol. 2012;115:79-83. doi: 10.1016/j.biortech.2011.10.073, PMID 22093978.
41. Rashid MM, Rahman MA, Islam MS, Hossen MA, Reza ASMA, Ahmed AMA, *et al*. Incredible affinity of Kattosh with PPAR-γ receptors attenuates STZ-induced pancreas and kidney lesions evidenced in chemicobiological interactions. J Cell Mol Med. 2022;26(12):3343-63. doi: 10.1111/jcmm.17339, PMID 35502486.
42. Rodríguez-Flores S, Escuredo O, Seijo MC. Characterization and antioxidant capacity of sweet chestnut honey produced in North-West Spain. J Apic Sci. 2016;60(1):19-30. doi: 10.1515/jas-2016-0002.
43. Prabhadevi V, Sahaya SS, Johnson M, Venkatramani B, Janakiraman N. Phytochemical studies on *Allamanda cathartica* L. using GC–MS. Asian Pac J Trop Biomed. 2012;2(2):S550-4. doi: 10.1016/S2221-1691(12)60272-X.
44. Chanda S, Baravalia Y, Nagani K. Spectral analysis of methanol extract of *Cissus quadrangularis* L. stem and its fractions. J Pharmacogn Phytochem. 2013;2(4):149-57.
45. Dey P, Dutta S, Chaudhuri TK. Comparative phytochemical profiling of *Clerodendrum infortunatum* L. using GC-MS method coupled with multivariate statistical approaches. Metabolomics. 2015;5(3):1000147.

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