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Original Article

ANTIOXIDANT, ANTIDIABETIC, ANTI-INFLAMMATORY AND ANTIFUNGAL ACTIVITY OF RAW AND COOKED STINKY BEANS (*PARKIA SPECIOSA*)

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ABSTRACT

Stinky beans (*Parkia speciosa*) is a traditional legume well-known for its uses as a medicine to treat various ailments. The objective of this research was to determine the presence of phytochemicals, estimate the total phenol and flavonoid content and assess the antioxidant activity, antidiabetic activity, anti-inflammatory activity and antifungal activity of ethanol extract of raw and cooked stinky beans seeds using in vitro assays. The phytochemicals namely terpenoids, phenolic compounds, flavonoids, tannins, glycosides, carbohydrates, saponins and proteins were present in both raw and cooked extracts, while alkaloids and saponins were not detected. Steroids was present in the raw extract but absent in the cooked extract. The total phenol content of raw stinky beans (93.17±8.18 mg GAE/g) was non-significantly higher than the cooked stinky beans (81.23±4.97 mg GAE/g) extract. On the other hand, the total flavonoid content of cooked stinky beans (81.74±1.34 mg QE/g) was significantly higher than the raw stinky beans (13.25±0.63 mg QE/g) extract. Both raw and cooked stinky beans possessed excellent scavenging and reducing activity. However, the cooked exert better antioxidant activity compared to the raw. On the other hand, the raw stinky beans exhibit better antidiabetic and anti-inflammatory activity compared to the cooked ones. Both raw and cooked stinky beans exhibited antifungal activity against *Candida krusei*, *Candida albicans* and *Candida tropicalis* organisms. The findings of this study indicate that stinky beans both in the raw and cooked form possess excellent health benefits and have high potential to be further explored in the world of nutrition and health.

KEYWORDS: Stinky beans, phytochemicals, antioxidant activity, antidiabetic activity, anti-inflammatory activity.

1. Introduction

Medicinal plants have been used since ancient time and are considered one of the leading treatments used in primary health care [1,2]. Different parts of plants like the leaf, flower, fruits, seeds, roots, and stems are usually utilized for medicinal purposes [1]. They can be used in the form of whole herbs or plants, syrups, teas, ointments, capsules and in powdered form [3]. The different parts of the plants contain phenolic and bioactive compounds such as alkaloids, anthocyanins, flavonoids, carotenoids, terpenoids and steroids which are known to exhibit antioxidant, anti-bacterial, anti-inflammatory, antidiabetic, anti-hypertension, anti-viral, anti-cancer and antifungal activities [4]. Amidst of advanced modern drugs available in today's world, medicinal or traditional medicine continues to thrive as a viable alternative offering unique approaches to healthcare due to its affordability, availability and effectiveness [2,5].

Stinky beans (*Parkia speciosa*) is a leguminous plant belonging to the family Fabaceae, well known for its traditional uses in treating different ailments. It is a long, flat green twisted pod which consists of 15-18 light green seeds with a foul and distinctive smell. Stinky beans are commonly grown in Southeast Asian countries like Malaysia, Indonesia, Thailand, Myanmar and Northeast part of India. Stinky beans can be eaten in raw, roasted, cooked or blanched form. The seeds can be used in making pickles, salads and added to seafoods, stir fried food and as an ingredient in traditional cuisines. Traditionally, the seeds are reported to be used in treating diabetes, headache, itchiness, inflammation, oedema, liver failure

and deworming. The seeds were also reported to be utilized in treating kidney diseases and urinary tract infections [6,7].

Various studies on raw stinky beans have been conducted on the nutraceutical, functional and health benefits using different form of extract and treatments. However, there is a notable gap in research with respect to the properties of raw and cooked stinky beans. Hence, this research aims to deepen our understanding of the properties of both raw and cooked stinky beans using in vitro study by assessing their antioxidant, antidiabetic, anti-inflammatory and antifungal properties.

2. Materials and Methods

Stinky beans were purchased from local market in Lamka, Churachandpur, Manipur, India.

The beans were prepared by thoroughly washing and soaking for 24 hours at room temperature. After soaking, the beans swelled up and the outer hard covering was removed from the seeds. As for the cooked sample, the beans after removing the outer covering were subjected to pressure cooking for about 15 minutes.

2.1. Extraction

The ethanol extract of stinky beans was prepared using maceration method. Both the raw and cooked stinky beans (50 g each) were ground using a grinder and soaked in 200 mL of ethanol for 72 hours at room temperature in a 250 mL sterile conical flask, sealed with a cotton plug. After 72 hours of the soaking process, the supernatant was filtered out using filter paper and the filtrate was then stored for 3 days in an air tight amber bottles in a refrigerator at 3° C for further analysis.

2.2. Phytochemical screening

Phytochemical analysis of both raw and cooked stinky beans extract was screened for the presence of alkaloids, terpenoids, steroids, phenolic compounds, flavonoids, tannins, glycosides, carbohydrates, saponins, steroids and proteins using the method given by Raaman and Tiwari [8,9].

2.2.1. Determination of total phenols

The total phenol content of stinky beans was determined using Folin-Ciocâlteu reagent method. One hundred µL of stinky beans extract was mixed with 900 µL of ethanol and 1 mL of Folin- Ciocâlteu reagent which was diluted in 10 mL of distilled water. Next, 1 mL of Na_2CO_3 (7.5 %, w/v) solution was added and shaken well. The mixture was then incubated in the dark at room temperature for 30 minutes. Then, the absorbance was measured by UV-VIS spectrophotometer at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (µg/mg of extract) [10].

2.2.2. Determination of total flavonoids

Total flavonoid content of cooked stinky beans extract was determined using aluminium chloride colorimetric method [11]. Five hundred µL of the stinky beans extract (1 mg/mL) was mixed with 500 µL of methanol. Then, 1 mL of 5 % (w/v) sodium nitrite solution and 1 mL of 10 % (w/v) aluminium chloride solution were added and shaken well. Next, 100 µL of 1 M NaOH solution was added, shaken well and subjected to incubation for 30 minutes at room temperature. The absorbance was measured at 510 nm and the result was expressed as (µg/mg of extract) quercetin equivalent.

2.3. Antioxidant activity

The antioxidant activity of both raw and cooked stinky beans was analyzed using DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay, superoxide radical scavenging assay, phosphomolybdenum reduction assay and ferric (Fe^{3+}) reducing power assay.

2.3.1. DPPH radical scavenging assay

The DPPH assay was used to evaluate the free radical scavenging activity of raw and cooked stinky beans [12]. Ascorbic acid was used as the standard reference. One mL of methanol was mixed with 1 mL of various concentration of the extracts (20-120 µg/mL). Next, 1 mL of DPPH reagent solution was added to the mixture and shaken well. Then the mixture was incubated in the dark for 30 minutes. For the control, 1 mL of ethanol was mixed with 1 mL DPPH solution. The decrease in absorbance was read at 517 nm using UV-VIS spectrophotometer. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{of DPPH inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \text{x}100$$

2.3.2. Superoxide (O_2^-) radical scavenging activity assay

Superoxide (O_2^-) radical scavenging assay was carried out based on riboflavin-EDTA-NBT assay method [13]. One mL of stinky beans (raw and cooked) extract at different concentrations (20-120 µg/mL) was mixed with 1 mL of methanol, 200 µL of riboflavin, 100 µL of EDTA (0.04 g in 10 mL buffer) and 50 µL of NBT solutions. Then, 1 mL of superoxide buffer was added which was prepared in 50 mM phosphate buffer with a pH of 7.6. Further, it was kept under UV for 90 seconds and the absorbance was immediately read at 590 nm by UV-VIS spectrophotometer. Ascorbic acid was used as the standard reference. The percentage inhibition was calculated using the following formula:

$$\%\ of\ \text{Superoxide radical inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \text{x}100$$

2.3.3. Phosphomolybdenum reduction assay

The antioxidant activity of raw and cooked stinky beans was also assessed by phosphomolybdenum reduction assay method [14]. The stinky beans extracts ranging from 20 µg/mL to 120 µg/mL concentration was mixed with 1 mL of methanol and 1 mL of phosphomolybdenun reagent containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). Then, the mixture was incubated in a water bath at 90°C for 90 minutes. Subsequently, the absorbance was measured at 695 nm using UV-VIS spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of inhibition was calculated using the following formula:

$$\%\ \text{of Phosphomolybdenum reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \text{x}100$$

2.3.4. Ferric (Fe^{3+}) reducing power assay (FRAP)

The reducing power of raw and cooked stinky beans was determined by ferric (Fe^{3+}) reducing power assay method given by Do et al. [15]. One mL of stinky beans extract at varying concentrations (20, 40, 60, 80, 100 and 120 µg/mL) was mixed with 1 mL of methanol, 1 mL of 1% (w/v) potassium ferricyanide [$K_3Fe(CN)_6$] solution and 1 mL phosphate buffer (0.2 M, pH 6.6) solution. The mixture was then incubated at 50 °C for 20 minutes in a water bath. Further, 500 µL of 10 % (w/v) trichloroacetic acid was added followed by 100 µL mixture of 0.1% (w/v) $FeCl_3$ and shaken well. The absorbance was measured at 700 nm by using UV-VIS spectrophotometer. Ascorbic acid was used as the standard reference. The percentage of reduction was calculated using the following formula:

$$\%\ (Fe^{3+})\text{of reduction} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \text{x}100$$

2.4. Antidiabetic activity

The antidiabetic activity of both raw and cooked stinky beans extract was examined using alpha amylase enzyme inhibition assay [16].

2.4.1. Alpha amylase enzyme inhibition assay

Alpha amylase enzyme inhibition assay was carried out using starch-iodine test method [16]. The total assay mixture was composed of various concentrations ranging from 20-120 µg/mL for both raw and cooked stinky beans. 20 µL of 1% (w/v) solution of alpha amylase enzyme prepared in 0.02 M sodium phosphate buffer with a pH of 7.0 containing 6 mM sodium chloride was added and incubated at 37 °C for 5 min. Then, 200 µL of soluble starch (1 %, w/v) was added to each reaction set and incubated at 37 °C for 60 min. Further, 100 µL of 1 M HCl was added to stop the enzymatic reaction followed by 200 µL of iodine reagent (5 mM I_2 and 5 mM KI). The colour change was noted and the absorbance was read at 595 nm. Acarbose and metformin was used as the standard reference.

$$\%\ \text{of}\ \alpha - \text{amylase enzyme inhibition} = \frac{\text{Sample} - \text{Control}}{\text{Sample}} \text{x}100$$

2.5. Anti-inflammatory activity

The anti-inflammatory activity of both ethanol extract of raw and cooked stinky beans was assessed using membrane stabilization assay method by Okoli et al. [17].

2.5.1. Membrane stabilization assay: preparation of Red Blood cells (RBCs) suspension

Blood was collected from a healthy human volunteer with no history of NSAIDs (Non-Steroidal Anti-Inflammatory Drugs) usage prior to 2 weeks of the experiment using vacutainer tube EDTA. It was then transferred to a centrifuge tube and centrifuged at 3000 rpm for 10 min and the pale-yellow plasma which appeared at the top layer was discarded. Then, about 10 mL of PBS was poured into centrifuge tube which was again centrifuged at 3000 rpm for 5 min, after which the supernatant was discarded and the washing process was repeated once again. The volume of the dissolved red blood pellets obtained was measured and reconstituted as a 10% v/v suspension with isotonic buffer solution using 10 mM sodium phosphate buffer with pH of 7.4.

2.5.2. Heat induced haemolysis

The reaction mixture (2 mL) consisted of 1 mL test sample of extract concentrations ranging 20-120 µg/mL and 1 mL of 10 % RBCs suspension, and for the control sample, only saline was added to the control test tube. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56 ºC for 30 min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. Aspirin was used as a standard reference. The percentage inhibition of haemolysis was calculated as follows:

$$\%\ \text{of hemolysis inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \text{x}100$$

2.6. Antifungal activity

2.6.1. Potato Dextrose Agar Medium

Potato dextrose agar medium was prepared according to the standard methods (200 g of potato, 20 g of dextrose, 1000 mL of distilled water and 20 g of agar). The required amount of each ingredient was weighed and suspended in the required volume of 125 mL of distilled water and then autoclaved at 15 lbs and 121ºC for 15 min. Next, the hot medium was poured in sterile petri plates which were already placed in the aseptic laminar chamber and the medium was allowed to solidify for 15 min.

2.6.2. Agar well diffusion method

Antifungal activity of raw and cooked stinky beans extract was carried out using agar well diffusion method. The solidified potato dextrose agar in the petri plates was inoculated by dispensing the inoculum using sterilized cotton swabs which are previously immersed in the inoculum broth in the test tube and evenly spread over to the solidified agar medium. After that, five wells were created in each plate using sterile well-borer (8 mm diameter) and the extracts were poured in each well containing 250, 500 and 1000 µg/mL concentrations. All the plates with the extracts loaded wells were incubated at 37ºC for 24 h and the antifungal activity was measured by the inhibition zone formed around the well. Fluconazole (50 µg) was used as the positive control [18]. The antifungal activity was performed against organisms such as *Candida krusei, Candida albicans* and *Candida tropicalis*.

2.7. Statistical analysis

The data were subjected to statistical analysis using One-way ANOVA. Tukey's post-hoc test was used for multiple comparison between groups with statistically significant at $p<0.05$ using R-software (R 4.4.1) version. Independent t-test was used for comparison of the means. GraphPad prism version 8 was used to plot the graphs and perform the independent t-test.

3. Results and Discussions

3.1. Phytochemical screening of raw and cooked stinky bean seeds

The phytochemical screening of raw and cooked stinky beans is presented in Table 1. The stinky beans

extracts were screened for the presence of phytochemicals such as alkaloids, terpenoids, phenolic compounds, flavonoids, tannins, glycosides, carbohydrates, saponins, steroids and proteins.

Table 1. Phytochemical screening of ethanol extract of raw and cooked stinky beans

Compounds	RSB	CSB
Alkaloid	-	-
Terpenoids	+	+
Phenolic compounds	+	+
Flavonoids	+	+
Tannins	+	+
Glycosides	+	+
CHO	+	+
Saponins	-	-
Steroids	+	-
Proteins	+	+

+:presence of phytochemical compounds, -: Absence of phytochemical compound

The presence of terpenoids, phenolic compounds, flavonoids, tannins, glycosides, carbohydrates and proteins were detected in both raw and cooked stinky beans while alkaloids and saponins were not detected. The presence of phytochemicals suggests antioxidant, reducing and radical scavenging activities. Steroids were present in the raw stinky beans but they were not detected in the cooked form. Similar results were also confirmed in studies done by Ghasemzadeh et al. [19] and Maria et al. [20] that reported the presence of terpenoids, phenolic compounds, and flavonoids and the absence of saponin and tannin in ethanol seed extract of stinky beans. In contrast to study conducted by Sonia et al. [21], the presence of alkaloids in stinky beans was not detected using ethanol extract. This suggests that the bioactive compounds identified also depend on the type of the extracts, such as methanol, ethanol, aqueous and chloroform used for the screening process. Additionaly, the phytochemicals present in a plant is also influenced by various factors such as geographical region, season, soil types and conservation methods [22].

3.2. Estimation of Total phenols and flavonoids

The quantitative estimation of total phenols and flavonoids in raw and cooked stinky beans is presented in Table 2.

Table 2. Estimation of total phenols and flavonoids of ethanol extract of raw and cooked stinky beans

Estimation of Phytochemicals	RSB	CSB
Total Phenols (mg GAE/g)	93.17±8.18a	81.23±4.97a
Total Flavonoids (mg QE/g)	13.25±0.63b	81.74±1.34c

Different superscripts in the same row indicate statistical significance ($p< 0.05$) using independent t-test. Data are presented as mean ± standard deviation from triplicate determination ($n = 3$). GAE - Gallic Acid Equivalent; QE - Quercetin Equivalent

Results showed that total phenol content in raw stinky beans (93.17±8.18 mg GAE/g) was non-significantly higher than the cooked stinky beans (81.23±4.97 mg GAE/g). According to Acito et al. [23], a small decline in the total phenolic content was reported when pressure cooked suggesting that cooking method influences the leaching of phenolic compounds. The finding of this study is consistent with studies conducted by Alide et al. [24] and Zhang et al. [25] that reported that the total phenol content decreased after subjected to cooking. This may also be attributed to the susceptibility of phenols to heat, that affect the structures leading to reduced content of phenolic compounds [25]. The total flavonoid content of raw stinky beans is 13.25±0.63 mg QE/g. In confirmation to this study, Ghasemzadeh et al. [19] reported that the total flavonoid content of stinky beans from different locations of Malaysia was 12.4 ± 3.5, 19.2 ± 1.49 and 7.4 ± 1.88 mg QE/g using ethanol extract which is in similar range to the present study. A study conducted by Balaji et al. [26] also reported that the total flavonoid content of stinky beans to be 14.16 ± 0.02 mg QE/g. However, in the present study, a higher total flavonoid content (81.74±1.34 mg QE/g) was found in the stinky beans extract after cooking. This is in agreement to other studies conducted by Boua et al. [27] and Bhave and Dasgupta [28] that also reported significant increase in total phenol content after exposure to different cooking process [27]. According to Gunathilake et al. [29] the cooking process enhanced the availability of flavonoids for extraction, increasing the release of the same from the altered cell structure.

3.3. Antioxidant activity

3.3.1. DPPH assay

The ability of both raw and cooked stinky beans to scavenge free radicals was assessed using DPPH assay and the percentage inhibition activity is presented in Fig. 1.

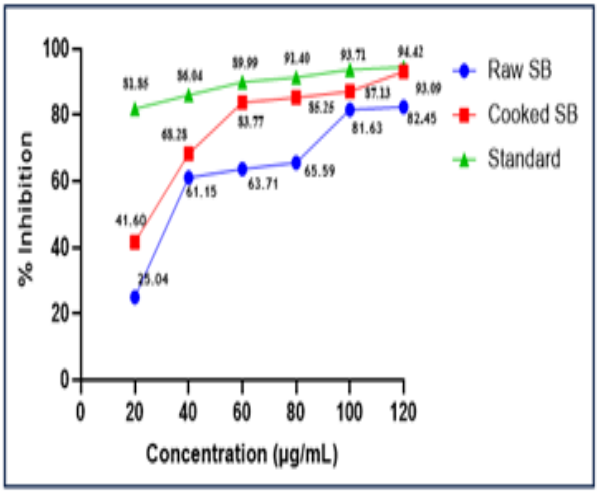

Fig. 1. Percentage inhibition activity of ethanol extract of raw and cooked stinky beans using DPPH radical scavenging assay

DPPH is an assay based on the capacity of antioxidants to scavenge DPPH radicals using spectrophotometric measurements. It is one of the most

commonly used method to determine antioxidant activity due to its simplicity and reproducible results [30]. The results demonstrate that the inhibition activity of both the raw and cooked stinky beans extracts significantly increased with the increasing concentration of the extract. The highest inhibition activity was recorded at 120 µg/mL, with 82.45% and 94.42% for raw and cooked stinky beans extract respectively. This is accounted to the content of phytochemicals and bioactive compouds present in the plant extract as the content of phenols is positively correlated to its antioxidant capacity [24].The IC_{50} of stinky beans using DPPH radical scavenging activity is presented in Table 3. The IC_{50} value showed that the standard has higher value followed by the cooked stinky beans and raw stinky beans. (Standard 12.22±9.92 µg/mL > Cooked 24.21±0.30 µg/mL > Raw 32.4±5.45 µg/mL). This highlights that the cooked stinky beans extract has better antioxidant capacity to scavenge free radicals than the raw stinky beans. This result is similar to the findings of Balaji et al. [27] who reported that stinky beans could serve as a natural antioxidant with an IC_{50} value of 35.40 µg/ml.

3.3.2. Superoxide (O_2^-) radical scavenging assay

The radical scavenging activity was assessed using Superoxide (O_2^-) radical scavenging assay and the percentage inhibition of raw and cooked stinky beans is presented in Fig. 2. The results demonstrated that the extracts exhibited increased activity with the increase in the concentration of the extracts in a dose-dependent manner (20-120 µg/mL) significantly ($p<0.05$). The maximum superoxide radical scavenging activity of stinky beans was observed to be 41.48 % and 83.90 % for the raw extract and cooked extract respectively at 120 µg/mL. Results suggest that the cooked stinky beans extract may scavenge superoxide radicals more effectively which may contribute to the prevention of oxidative stress resulting changes and eventually diseases.

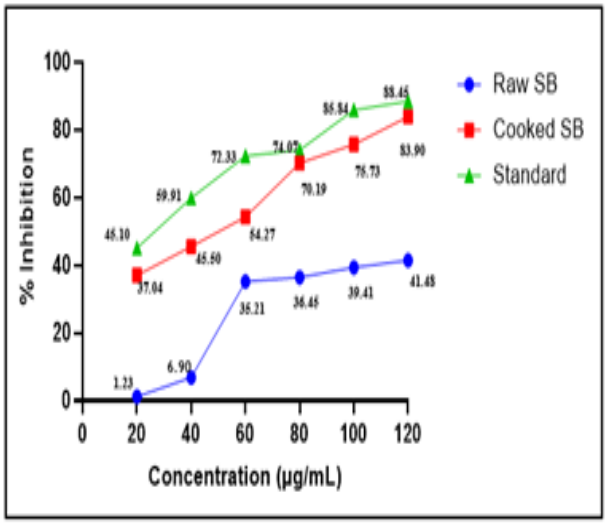

Fig. 2. Percentage inhibition of ethanol extract of raw and cooked stinky beans using Superoxide (O_2^-) radical scavenging assay

The IC_{50} of raw and cooked stinky beans extract using Superoxide (O_2^-) radical scavenging activity was compared to a standard (ascorbic acid) and is presented in Table 3. Results showed that the standard has the highest activity followed by the cooked and the raw stinky beans extract. (Standard 22.19±0.69 µg/mL > Cooked 52.32±8.10 µg/mL > Raw 144.99±0.61 µg/mL). This indicated that the cooked stinky beans exhibited better antioxidant activity than the raw stinky beans extract. This may be influenced by the cooking process that allows the release of more bound phenolic acids from the breakdown of the cellular constituents and increase the bioavailabitlity and effectiveness of bioactive compounds to scavenge free radicals [31].

3.3.3. Phosmomolybdenum reducing assay

The antioxidant activity of raw and cooked stinky beans was assessed using phosmomolybdenum assay. The percentage inhibition activity is presented in Fig. 3. This method is based on the reduction of Mo (VI) to Mo (V) by the formation of green phosphate Mo (V) complex. The percentage reduction activity of phosphomolybdenum assay reported that for all the extracts the reduction capacity increased as the concentration increases with highest inhibition at 120 µg/mL concentration. The maximum phosphomolybdenum reducing assay activity was observed at at 120 µg/mL with a percentage of 97.32 % and 95.10 % for raw and cooked stinky beans extract respectively.

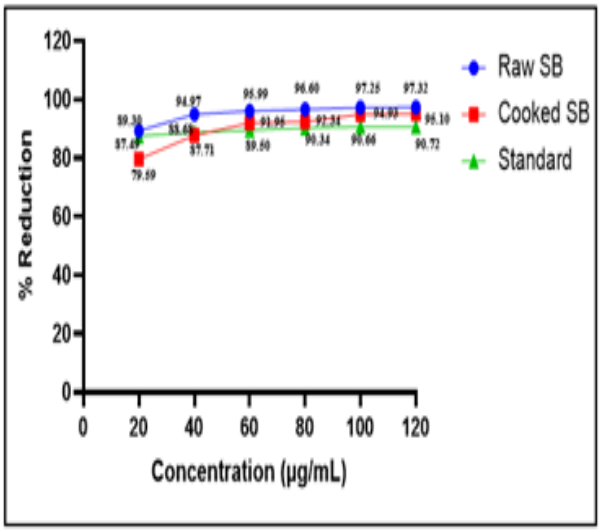

Fig. 3. Percentage reduction of ethanol extract of raw and cooked stinky beans using Phosphomolybdenum reduction assay

The RC_{50} of stinky beans using phosphomolybdenum is presented in Table 3. The reducing power activity showed that the raw stinky beans extract has the highest activity followed by the standard and the cooked stinky beans extract (Raw 11.20±0.96 µg/mL > Standard 11.43±0.76 µg/mL > Cooked 12.56±0.13 µg/mL). This may be because the raw stinky beans extract has the ability to retain better bioactive compounds which get diminished when subjected to cooking process [32,33]. This indicates that both extracts possessed excellent antioxidant reducing capacity. Moreover, raw stinky beans extract with an RC_{50} value of 11.20±0.96 µg/mL ($p<0.05$) showed lower value possessing highest antioxidant reducing capacity. This is attributed to the

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Table 3. IC_{50} value of ethanol extract of raw and cooked stinky beans for antioxidant activity

Extract/Sample	IC_{50} (µg/mL)		RC_{50} (µg/mL)	
	DPPH	Superoxide (O_2^-)	Phosphomolybdenum	FRAP
Standard	12.22±9.92	22.19±0.69	11.43±0.76	30.42±0.19
RSB	32.4±5.45	144.99±0.61	11.20±0.96	128.12±0.50
CSB	24.21±0.30	52.32±8.10	12.56±0.13	120.19±0.61

ability of raw stinky beans extract to reduce molybdenum ions efficaciously when compared to cooked stinky beans and the standard (ascorbic acid).

3.3.4. FRAP Assay

The reducing capacity of raw and cooked stinky beans was measured using FRAP assay. The percentage reduction activity of stinky beans extract is presented in Fig. 4. The reduction capacity of the extracts increases significantly ($p<0.05$) with the increase in the dose of concentrations with highest reduction recorded at 46.83 % for raw extract and 49.92 % for the cooked extract respectively. This increase in the percentage reduction indicates that the compounds present in stinky beans extract has the capacity to reduce Fe^{3+} to Fe^{2+} by donating an electron.

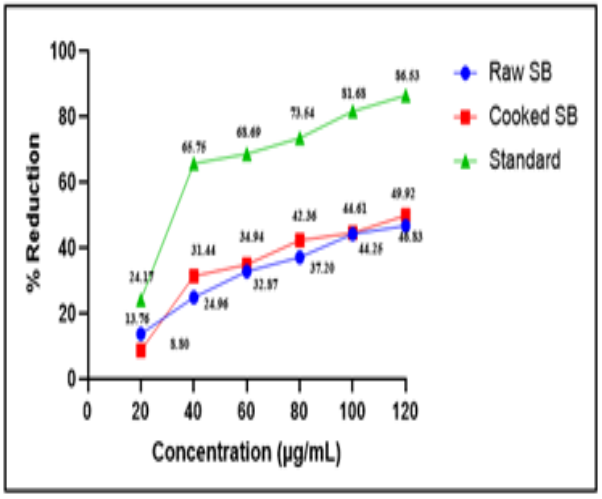

Fig. 4. Percentage reduction of ethanol extract of raw and cooked stinky beans using ferric (Fe^{3+}) reducing power assay

The RC_{50} of stinky beans using FRAP is presented in Table 3. Results showed that the standard showed highest activity followed by the cooked and raw stinky beans extract. (Standard 30.42±0.1961 µg/mL > Cooked stinky bean 120.19±0.61 µg/mL > Raw stinky bean 128.12±0.50 µg/mL). This suggests that cooked stinky beans have better ability to reduce ferric ions to ferrous ions compared to the raw stinky beans extract.

3.4. Antidiabetic activity

3.4.1. Alpha amylase enzyme inhibition assay

The antidiabetic activity of both raw and cooked stinky beans is assessed using alpha amylase enzyme inhibition assay. The alpha amylase enzyme is an enzyme that has a crucial role in the digestion of carbohydrates. The inhibition activity of alpha amylase enzyme is one way of indicating whether the extract has the potential to regulate blood glucose level. The inhibition activity is presented in Fig. 5. The inhibition activity of alpha amylase enzyme increased non-significantly with the increase in the dosage of the concentration. The maximum alpha amylase enzyme inhibition activity for the raw stinky beans was observed to be 37.73 % and 32.56 % for the cooked stinky beans respectively at 120 µg/mL.

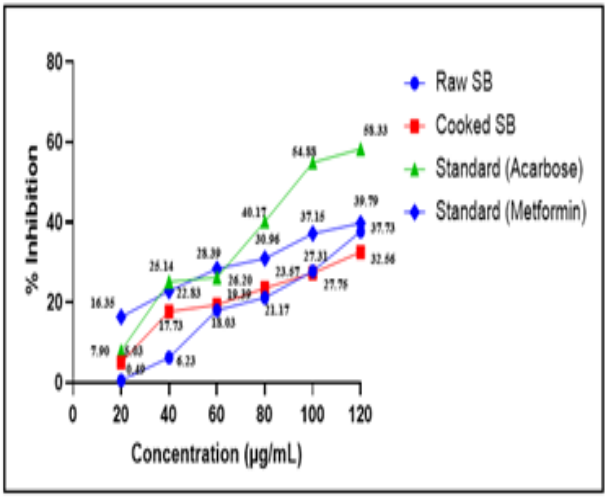

Fig. 5. Percentage inhibition of raw and cooked stinky beans using alpha amylase enzyme inhibition assay

IC_{50} is the concentration of the extracts needed to inhibit the alpha amylase enzyme inhibition activity. Lower values of IC_{50} indicate higher antidiabetic activity potential. The IC_{50} of stinky beans was compared to two standards, acarbose and metformin and the value is presented in Table 4. The raw stinky bean seeds when compared to the cooked stinky bean seeds showed non-significantly better antidiabetic activity which may be influence by the cooking processes suggesting that cooking diminished the bio-availability of bioactive compounds responsible for antidiabetic properties [34]. The IC_{50} of stinky bean seeds extract showed that the standard showed better antidiabetic activity followed by the raw and cooked extracts. (Acarbose 91.15±2.10 µg/mL > Raw 159.04±0.96 µg/mL > Cooked 184.28±1.45 µg/mL; Metformin 150.83±2.95 µg/mL > Raw 159.04±0.96 µg/mL > Cooked 184.28±1.45 µg/mL).

Table 4. IC_{50} value of raw and cooked stinky beans for antidiabetic activity

Extract/Standard	IC_{50} (µg/mL)
Standard (Acarbose)	91.15±2.10
Standard (Metformin)	150.83±2.95
Raw Stinky Bean	159.04±0.96
Cooked Stinky Bean	184.28±1.45

3.5. Anti-inflammatory activity

3.5.1. Human red blood cell stabilization assay (HRBC)

The anti-inflammatory activity was assessed using HRBC stabilization assay which is based on the principle that the extracts can stabilize the red blood cell membrane against haemolysis caused by heat induced stress. The anti-inflammatory activity percentage inhibition is presented in Fig. 6. The anti-inflammatory activity of both raw and cooked stinky beans increases with the increasing concentration significantly ($p<0.05$) from 20 to 120 µg/mL concentrations. The maximum percentage of inhibition of stinky beans was observed at 120 µg/mL with an inhibition percentage of 85.08% for the raw extract and 49.71% for the cooked extract, respectively.

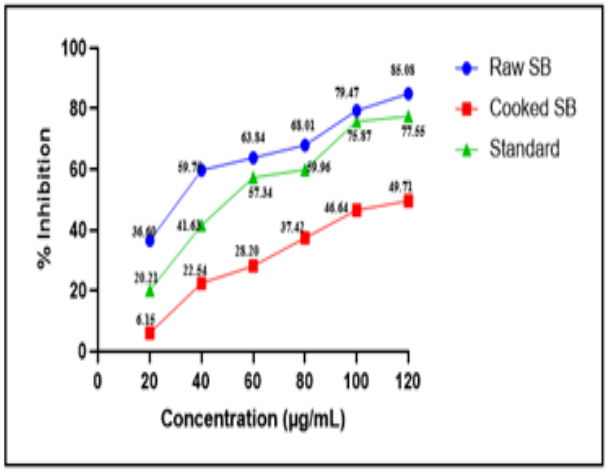

Fig. 6. Percentage inhibition of ethanol extract of raw and cooked stinky beans using HRBC stabilization assay

The IC_{50} of stinky beans using HRBC is presented in Table 5. Results showed that raw stinky beans extract exhibit significantly ($p<0.05$) better anti-inflammatory activity than the cooked stinky beans extract and the standard. (Raw 33.57±2.20 µg/mL > Standard 46.27±3.59 µg/mL > Cooked 121.29±10.26 µg/mL). This is influenced by the presence of phytochemicals and bioactive compounds responsible for the anti-inflammatory activity [35]. When cooked, the effectiveness of the beans extracts becomes lower than the raw extracts which could be because of the cooking process involved that might have altered the bioavailability of bioactive compounds.

Table 5. IC_{50} value of standard, raw and cooked stinky beans for anti-inflammatory activity

Extract/Standard	IC_{50} (µg/mL)
Standard (Aspirin)	46.27±3.59
Raw Stinky beans	33.57±2.20
Cooked Stinky beans	121.29±10.26

3.6. Antifungal activity

3.6.1. Agar wall diffusion assay

The antifungal activity of raw stinky beans is presented in Table 6. The maximum inhibition for raw stinky beans was demonstrated against *Candida tropicalis* (13 mm), *Candida albicans* (12 mm), and *Candida krusei* (10 mm). However, the zone of inhibition was observed only at 1000 µg concentration suggesting that higher dose of the extract is required to achieve its effectiveness. The standard fluconazole does not exhibit zone of inhibition suggesting that raw stinky beans extract has better antifungal activity compared to standard drugs fluconazole and has the potential to be used as antibiotic to treat infections.

The anti-fungal activity of cooked stinky beans is presented in Table 7. Results showed that cooked stinky beans extract acts as an excellent antifungal agent with maximum inhibition against *Candida krusei* (17 mm), *Candida tropicalis* (13 mm) *and Candida albicans* (10 mm) at 1000 µg concentration. This indicates that cooked stinky beans extract has antifungal potential against all organisms while indicating that higher concentration levels of the extract is needed to inhibit all organisms which aligns with other antifungal studies where higher concentration usually exhibits better efficacy [36,37]. This could be influenced by elevated levels of bioactive compounds present at higher dosage of the extract concentration promoting better antifungal inhibition activity [37].

Table 6. Antifungal activity of ethanol extract of raw stinky beans

Organism	Inhibition zone (mm)			
	250 µg	500 µg	1000 µg	Standard
Candida albicans	-	-	12 mm	-
Candida tropicalis	-	-	13 mm	-
Candida krusei	-	-	10 mm	-

Table 7. Antifungal activity of ethanol extract of cooked stinky beans

Organism	Inhibition zone (mm)			
	250 µg	**500 µg**	**1000 µg**	**Standard**
Candida albicans	-	-	10 mm	12 mm
Candida tropicalis	-	10 mm	13 mm	17 mm
Candida krusei	-	-	17 mm	12 mm

4. Conclusions

The findings of this study revealed that raw and cooked stinky beans are excellent sources of phytochemicals and possessed antioxidant, antidiabetic, anti-inflammatory and antifungal properties. Cooked stinky beans showed significant antioxidant activity for DPPH assay, superoxide radical scavenging assay and FRAP assay except for phosphomolybdenum assay, where the raw stinky beans extract showed significant activity compared to the cooked extract. With respect to antidiabetic activity the raw stinky beans possessed non-significantly higher antidiabetic activity than the cooked stinky beans. Similarly, the anti-inflammatory activity of raw stinky beans possessed significant activity compared to the cooked stinky beans. Regards to the antifungal activity, both raw and cooked stinky beans extract demonstrated antifungal activity against *Candida albicans*, *Candida tropicalis* and *Candida krusei* organisms. This suggests that stinky beans can be used as natural antioxidant, antidiabetic, anti-inflammatory and antifungal agent. It is recommended that future studies should focus on the bioactive compounds, the mechanisms involved, toxicity study, clinical trials and human supplementation study.

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