Evaluation of the Bioactive Compounds Present in Peanut Skin (*Arachis hypogaea* L.) for Antioxidant and Antifungal Activity

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Studies on peanuts (*Arachis hypogaea* L.) have shown that this food is rich in beneficial substances such as phenolic compounds and flavonoids. Peanut skin, the reddish-pink layer that surrounds the seed, is considered a by-product of processing and has a rich nutritional composition, containing a variety of phenolic compounds, including resveratrol and flavonoids, especially proanthocyanidins and their derivatives, which are predominant. These compounds have high antioxidant capacity and are able to neutralize free radicals, reducing oxidative stress associated with several chronic diseases. Some phenolic compounds also exhibit antifungal activity by inhibiting cell wall synthesis or altering fungal membranes. This study aimed to evaluate the presence and activity of phenolic compounds in peanut skin, focusing on antioxidant and antifungal properties. Ethanol extracts were tested through spectrophotometric quantification of total phenolics, flavonoids, and tannins, antioxidant activity using the DPPH method, and antifungal activity via agar disk diffusion against *Fusarium* sp. The extract showed strong antioxidant activity and high levels of phenolic compounds but limited antifungal activity. These findings support the potential of peanut skin compounds in food and pharmaceutical applications.
Keywords: Peanut Skin. Phenolics. Antioxidant. *Fusarium* sp.

Peanut (*Arachis hypogaea* L.) is a legume native to South America, cultivated in tropical and subtropical countries. One of the by-products generated after its processing is the peanut skin.

The composition of peanuts varies according to climate and soil conditions, consisting mainly of 41% carbohydrates, 10 to 20% lipids, 19% proteins, and 18% fiber. Peanut skin, in turn, is composed of a variety of phenolic compounds, such as resveratrol and flavonoids, with high antioxidant capacity and the ability to inhibit enzymes that are precursors of disorders and diseases [1].

Research on new natural bioactive substances has increased significantly in recent years, and their various health benefits are being increasingly elucidated and disseminated. Among these substances, antioxidants stand out, compounds capable of combating oxidative damage caused by free radicals, with wide applications in the pharmaceutical, cosmetic and food industries [2].

Brazil's economy is strongly based on agribusiness, which generates a large volume of byproducts that can harm the environment. However, according to studies, peanut skin has potential for the development of new products in various fields. Therefore, the main objective of this study is to evaluate the bioactive compounds present in peanut skin and their antioxidant potential and antifungal activity against the fungus *Fusarium* sp.

Materials and Methods

The present study is a qualitative-quantitative exploratory study, employing procedures such as literature review and experimental research.

Preparation of Peanut Skin Extract (*Arachis hypogaea* L.)

The peanut skins (*Arachis hypogaea* L.) used in this study were provided by the cereal company NP Zanchetta, located in Borborema, São Paulo, Brazil.

Received on 18 February 2026; revised 23 April 2026.
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J Bioeng. Tech. Health 2026;9(5):436-442
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The extract was prepared by the maceration process, at a ratio of 1:3.5 (dry mass:solvent), using ethyl absolute alcohol for 48 hours [3]. The pure ethanolic extract (EE) was obtained through simple distillation.

The extraction percentage yield was calculated using the equation 1, proposed by Xiao and colleagues [4], where the yield (%) is obtained by dividing the mass of the pure extract (mo), in grams, by the mass of the dehydrated biomass (mf) of derived from the agro-industrial residue of peanut skin, and then multiplying the result by 100 to obtain the percentage:

$$Yield(\%) = mo/mf \times 100 \qquad (1)$$

Quantification of Total Phenolics

Total phenolic compounds (TPC) were evaluated using the spectrophotometric method with 6% Folin–Ciocalteu reagent [5], with modifications. A standard curve was established using different concentrations of gallic acid (20 to 100 mg/L).

Four different concentrations (100, 10, 1, and 0.1 mg/mL) of the ethanolic extract from peanut skin were tested.

To perform the analysis, 0.25 mL of each extract concentration was pipetted into test tubes, followed by 2.75 mL of Folin–Ciocalteu reagent. The mixture was homogenized, and after 5 minutes, 0.25 mL of 10% sodium carbonate was added.

After 1 hour of incubation protected from light, the absorbance was measured at 755 nm using a spectrophotometer. All assays were performed in triplicate.

Flavonoid Quantification

The determination of total flavonoids was carried out, with modifications, according to the method described by Silva and colleagues [6]. Aliquots of 500 µL from different concentrations (100, 10, 1, and 0.1 mg/mL) of the ethanolic extract were added to analytical-grade methanol and a 2% methanolic solution of aluminum chloride ($AlCl_3$). After 30 minutes, the absorbance was measured at 415 nm using a spectrophotometer. All assays were performed in duplicate.

Quantification of Tannins

The quantification of total tannin content was performed using the method described by Pansera and colleagues [7], with modifications, using tannic acid as the standard. Two different concentrations (0.008 and 0.08 mg/mL) of the ethanolic extract were used.

To each 1 mL aliquot of extract, 1 mL of Folin–Ciocalteu reagent was added. Then, 1 mL of 8% sodium carbonate solution was added to the mixture, which was shaken and left to rest for 2 hours, protected from light. After this period, the absorbance was measured at 725 nm using a spectrophotometer. All assays were performed in duplicate.

Antioxidant Activity

Antioxidant activity was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method, as described by Rufino and colleagues [8], with modifications. For this procedure, a previously prepared DPPH solution was used, along with the test substance at four different concentrations (100, 10, 1, and 0.1 mg/mL). In this assay, 50% methanol and 70% acetone were also used as reference substances.

Each determination consisted of 3.9 mL of DPPH solution and 100 µL of the test concentration. The control reaction (blank) was performed using 4 mL of methanol. Absorbance readings were taken at 515 nm after 45 minutes of incubation at room temperature, protected from light, using a spectrophotometer. All assays were performed in duplicate.

Antioxidant activity (AA) was expressed according to equation 2, where Abs_{DPPH} is the initial absorbance of the 60 mM DPPH solution,

and Abs_{sample} is the absorbance of the reaction mixture [5].

$$AAT(\%) = \frac{\{[Abs_{DPPH} - (Abs_{sample} - Abs_{blank})]x100\}}{Abs_{DPPH}} \quad (2)$$

Agar Disk Diffusion Test

To evaluate the antifungal capacity of the ethanolic extract, tests were conducted using the agar disk diffusion method. A subculture of the main strain was made on Petri dishes containing PDA medium (Potato Dextrose Agar), previously sterilized in an autoclave (121 °C, 1 atm).

On each plate with the *Fusarium* sp. subculture, three sterile filter paper discs were placed 3 cm apart from each other and 1 cm from the edge of the plate. Then, 10 µL of the extract concentration was applied to each disc. The ethanolic extract was tested at two concentrations: 10 and 100 mg/mL. A control test was also performed to evaluate the effect of ethanol on the fungus, applying 10 µL of ethanol per disc. In the negative control, the fungus was not exposed to any degrading agent.

The disc tests were performed in triplicate, and all assays were duplicated.

The plates were incubated in a bacteriological oven at 28 °C for 7 days. After fungal growth, results were collected by measuring the diameter of the mycelium. The percentage of mycelial growth inhibition was calculated according to França [9], where dc is the average diameter of the control and dt is the average diameter of the fungus treated with the ethanolic extract (Equation 3).

$$\%mycelial\ inhibition = (dc\text{-}dt / dc) \times 100 \quad (3)$$

Results and Discussion

The obtained data reveal that the yield of the ethanolic extract of peanut skin (EEPS) was 2.8% in relation to the total biomass. The process for obtaining this value is shown in Table 1.

Quantification of Total Phenolics

At the concentration of 0.1 mg/mL, a total phenolic content (TPC) of 2.26% was observed. The 1 mg/mL concentration showed a TPC of 6.06%. At 10 mg/mL, the TPC reached 7.16%, and the highest percentage was observed at 100 mg/mL, with a TPC of 36.86%, as shown in Table 2.

The data indicates that increasing the concentration of the extract is associated with a higher total phenolic content, as illustrated in Figure 1, which compares the TPC values at each concentration analyzed.

A study also evaluated the phenolic content in peanut skin extracts using 80% ethanol as the solvent [10]. The TPC of the 25 mg/mL extract concentration from the IAC Runner 886 and IAC-Tatu-ST peanut varieties was determined, with values of 0.5438 mg GAE/g and 0.6478 mg GAE/g, respectively. These values are lower than those obtained in the present study, which may be attributed to the extraction procedure, evaporation techniques, and the concentrations used.

These findings highlight the importance of experimental conditions and confirm the potential of analytical-grade ethanol (P.A.) as a solvent to enhance the extraction of target compounds.

Determination of Flavonoids

Flavonoid content was expressed as a percentage of quercetin equivalence (QE). At the

Table 1. Representation of the calculated results using Formula 1.

Ethanolic Extract	Extract Yield in Grams	Dehydrated Biomass in Grams	Extraction Percentage
EEPA	16.85g	600g	2.80%

Table 2. Representation of total phenolic content in concentrations.

[] mg/mL	[] AG/mL	% Phenolic Content
0.1	3,596	2,266
1	9,626	6,066
10	11,371	7,166
100	58,499	36,866

Figure 1. Percentage of phenolic content at concentrations of 0.1, 1, 10 and 100mg/mL.

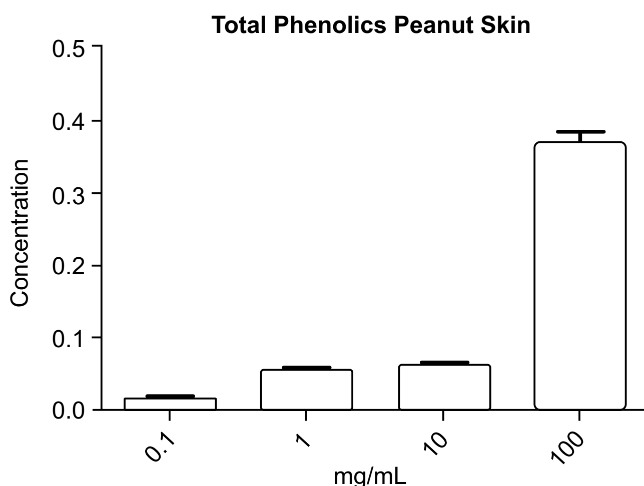
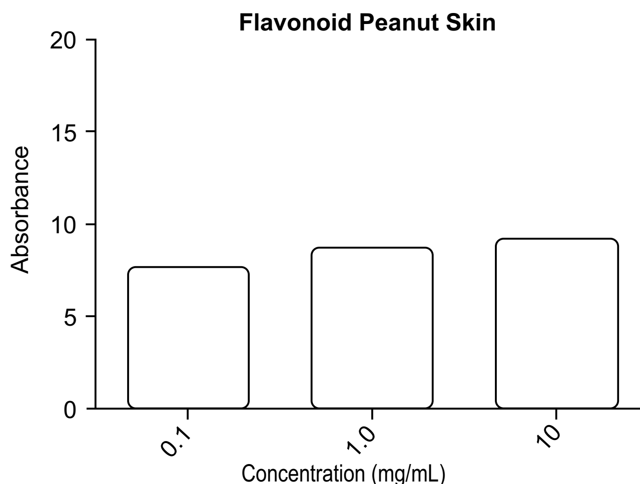


Table 3. Representation of flavonoids in concentrations.

[] mg/mL	QE %
0.1	7.70
1	8.70
10	9.20
100	177.70

Figure 2. Percentage of flavonoids at concentrations of 0.1, 1 and 10mg/mL.



concentration of 0.1 mg/mL, the value obtained was 7.7% QE; at 1 mg/mL, it was 8.7% QE. For the 10 mg/mL concentration, the value was 9.2% QE. At 100 mg/mL, the value reached 177.7% QE, representing an exponential increase, as shown in Table 3 and illustrated in Figure 2, which compares the flavonoid content at each concentration.

The results obtained can be compared to those presented in another study [11], which employed a colorimetric method to evaluate the presence of flavonoids in eight legumes, reporting values ranging from 135 to 191 mg QE/g, which are higher than the data obtained in this study.

This difference may be related to several factors, such as the concentration analyzed, the solvent used for bioactive compound extraction, and the legume species.

Quantification of Total Tannins

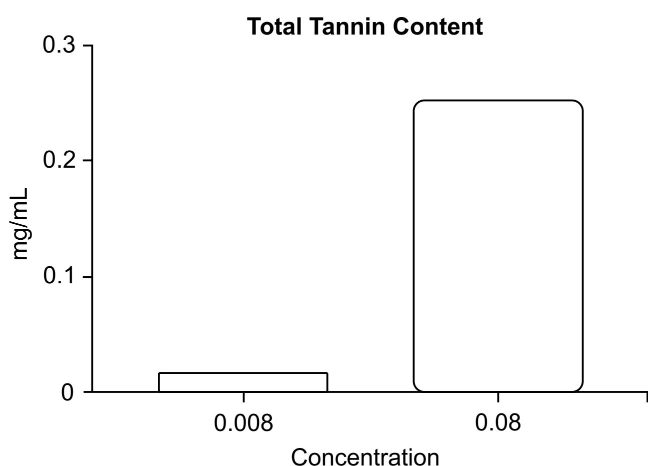
At the concentration of 0.008 mg/mL, a total tannin content (TTC) of 0.017% was obtained, indicating a lower tannin level. At the concentration of 0.08 mg/mL, the TTC was 0.253%, demonstrating a considerable increase in tannin content at higher concentrations, as shown in Table 4 and Figure 3, which compares tannin levels at each concentration.

The results obtained can be compared with the values reported by Benevides and colleagues [12],

Table 4. Representation of tannins in concentrations.

[] mg/mL	TTP %
0.008	0.017
0.08	0.253

Figure 3. Percentage of tannins at concentrations of 0.008 and 0.08 mg/mL.



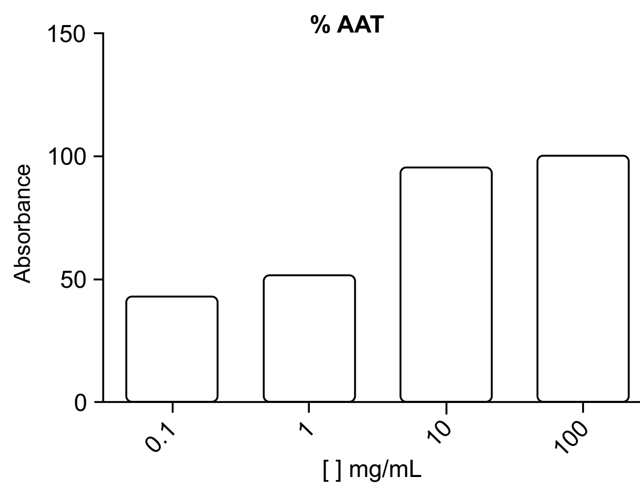
who evaluated the tannin content in legumes such as green beans and pigeon peas, finding values of 4.71 mg/100 g and 6.05 mg/100 g, respectively. These values are higher than those found in the present study, which may be attributed to differences in legume species and the method used for the quantification of this bioactive compound.

Antioxidant Activity of Peanut Skin Extract (*Arachis hypogaea* L.)

The results demonstrated that the peanut skin extract exhibits significant antioxidant activity at all tested concentrations (0.1, 1, 10, and 100 mg/mL). The 100 mg/mL EEPS concentration reached 95.7%, while the 10 mg/mL concentration achieved 91.8%, followed by 55.6% at 1 mg/mL, and 48% at 0.1 mg/mL. These values demonstrate an excellent antioxidant capacity of peanut skin, even at low concentrations, as illustrated in Figure 4.

When analyzing the data from this study alongside a research conducted by Diniyah and

Figure 4. Percentage of antioxidant activity at concentrations of 0.1, 1, 10 and 100 mg/mL.



colleagues [13], which provides information on antioxidant activity in non-oilseed legumes, specifically *Phaseolus lunatus* and *Canavalia ensiformis*, it was shown that for the analyzed seeds, the amount required to reduce the initial DPPH concentration ranged from 1.83% to 19.42%.

These value variations may be related to several factors, such as the legume species, the extraction method of the target compound, and the solvent used.

Antifungal Activity of Extract on *Fusarium* sp.

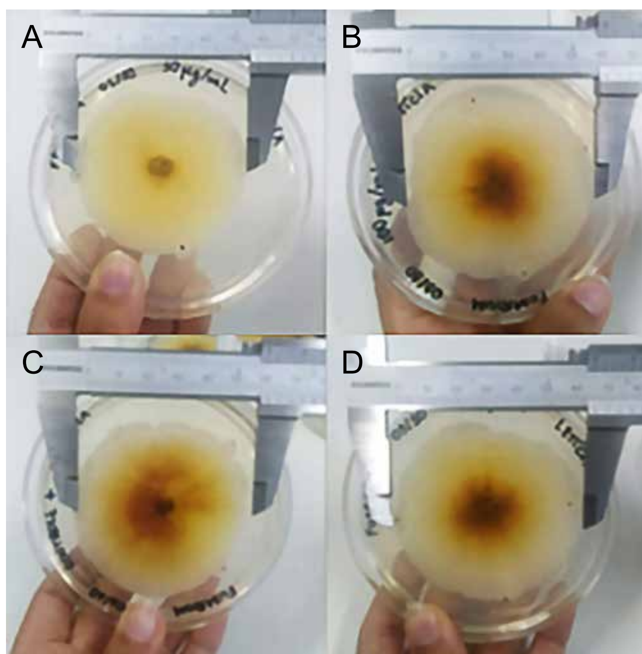
After 7 days of incubation, mycelial growth was measured under treatment with the peanut skin extract. At the 10 mg/mL, an inhibition rate of 17.17%. However, at 100 mg/mL, the lowest inhibition percentage was recorded, at 13%, as shown in Table 5. Figure 5 displays images of mycelial measurements on the 7th day, along with the negative control and the solvent test, which is essential for evaluating the direct impact on fungal growth.

The average measurement of the negative control was conducted in duplicate, resulting in a value of 69 mm. The ethanol assays showed a moderate effect on fungal growth, exhibiting an inhibition rate of 14.13%.

Table 5. Representation of data from different concentrations of peanut skin extract under *Fusarium* sp. The mycelial mean value was calculated from a duplicate test.

Sample	Concentration	Mycelial Inhibition (%)
Peanut skin extract	10 mg/mL	17.17%
Peanut skin extract	100 mg/mL	13.0%
Ethanol Control	100%	14.13%
Negative Control	-	0 %

Figure 5. Mycelial growth measurement of *Fusarium* sp. isolate under treatment with ethanolic extract of peanut skin after 7 days of incubation. A) Peanut skin extract 10 mg/mL; B) Peanut skin extract 100 mg/mL; C) Ethanol control; D) Negative control.



Although the extract demonstrated some inhibitory capacity, the mycelium was able to proliferate on the discs subjected to this treatment, which may be associated with several factors such as fungal resistance to the bioactive compounds.

In a study that investigated the antimicrobial effects of *Dioclea grandiflora* Mart. ex Benth, it was observed that the ethanolic extract of this plant did not exhibit inhibitory activity against *Fusarium* sp., whereas the hydroalcoholic extract showed a satisfactory response against dermatophytes [14].

This highlights the importance of evaluating different extraction methods and solvents to increase the amount of bioactive compounds and enhance antifungal activity.

Conclusion

This study showed that peanut skin (*Arachis hypogaea* L.) contains a significant amount of phenolic compounds, flavonoids, and tannins, attributing remarkable antioxidant properties to this by-product. It was observed that antioxidant activity is directly related to the concentration of the ethanolic extract, reaching values above 90% at higher concentrations. This highlights the extract's ability to neutralize free radicals.

Regarding antifungal activity, the results indicated limited inhibition against *Fusarium* sp. Future research may investigate solvent combinations or improvements in extraction techniques to increase the yield of compounds with antifungal properties and also explore their application in food and pharmaceutical systems, expanding their impact on health and sustainability.

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