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A Study on the Antioxidant and Antimicrobial Activities of Seed and Leaf Extracts of *Glycine max* (L) Merr.

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Authors' contributions

This work was carried out in collaboration between both authors. Authors CVI and NAI designed the study and wrote the protocol. Both authors wrote the first draft of the manuscript. Both authors managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to evaluate the antioxidant and antimicrobial activities of ethanolic leaf and seed extracts of Soybean (*Glycine max* (L) Merr.). Antioxidant activity of the leaf and seed extracts was determined by reducing power capacity of flavonoid, phenol, lycopene, β-carotene and ascorbic acid using standard laboratory method. The antimicrobial assay of the leaf and seed extracts was carried out at different concentrations against some selected clinical pathogens (bacterial strains: *Staphylococcus aureus, Escherichia coli;* fungi strain*: Candida albicans, Aspergillus niger*) using agar diffusion method. Analysis of variance was employed in data analysis. Antioxidant phytochemical composition of the leaf and seed extracts of *Glycine max* revealed that all the phytochemicals assayed were present but in various amounts except ascorbic acid that was absent in the leaf. The quantities of the phytochemicals were higher in the leaf than in the seed except for lycopene that was higher in the seed and ascorbic acid that was present only in the seed. The Reducing Power Capacity of the ethanol extract of the leaf and seed of the *Glycine*

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max indicated that *Glycine max* extracts though had a reducing power capacity but is very low in comparison with the standard used. The effectiveness of the extracts increases slightly as the concentration increases. Antimicrobial studies indicated that the ethanolic leaf and seed extracts of *Glycine max* inhibited the growth of the investigated microbes and the inhibition was concentration dependent. The leaf extract showed higher inhibition than the seed. However, the ethanolic leaf and seed extracts showed no inhibition against bacterial strains at 50 mg/ml. Data obtained from the studies showed that the plant possessed antioxidant and antimicrobial properties and could be used in the treatment of microbial infections especially fungi infections and also to protect cells against free radical and excessive light damages. However, the leaf extract showed better inhibition than the seed extract indicating that it is a better antimicrobial agent than the seed.

Keywords: Antimicrobial; antioxidant; bioactive compounds; Glycine max.

1. INTRODUCTION

Soybean (*Glycine max* (L.) Merr) is one of the world's most essential seed legumes, which contributes 25% to the global edible oil. Soybean demand has continued to increase more than other crops due to its high protein and oil contents [1]. It is an important oilseed belonging to the family Leguminosae and usually cultivated as food crop [2]. Soybean importance includes milk production, oil processing, livestock feeds, industrial uses and human consumption. Soybean has been recognised to be an ideal grain for meeting protein and energy requirements of both man and animal. It is probably one of the world's most valuable crops, used as feed by billions of livestock, as a source of dietary protein, oil and in the industrial manufacture of several products [3,4]. Soybean is an extremely rich source of protein and fat, and a good source of energy, vitamins and minerals with an average production cycle of 90-110 days from planting to harvesting. Of all plant foods consumed globally, it is ranked number one as the richest in food value [5]. It is very useful in improving the menu of malnourished children and revitalising heart and breast cancer patients, and it has no cholesterol. Soybean can be used as a nutritional supplement for pregnant women, lactating mothers and children [6].

Various studies have been published, investigating the antimicrobial and antioxidative activities of plant-derived compounds against a range of pathogens, as well as for the discovery of new antioxidant and antimicrobial compounds [7,8,9] and [10]. Antioxidants are phytochemicals, vitamins and other nutrients that protect our cells from damage caused by free radicals. *In vitro* and *in vivo* studies have shown that antioxidants help prevent the free radical damage that is associated with cancer and heart disease. Antioxidants can be found in most fruits and vegetables but also culinary herbs and medicinal herbs can contain high levels of antioxidants [11]. Antimicrobial agents are substances that kill or inhibit the growth of microorganisms such as bacteria, fungi and protozoans. Antimicrobial compounds derived from plants might inhibit bacteria through different mechanisms and provide clinical values for the treatment of infections caused by resistant microbes [8].

Despite the tremendous benefits derivable from soybean, there has not been quiet enough information on antioxidant and antimicrobial potentials of its various parts, especially in South Eastern Nigeria hence the need for this study. The objective of this research was to evaluate the antioxidant and antimicrobial activities of the seed and leaf extracts of *Glycine max*.

2. MATERIALS AND METHODS

2.1 Study Area, Collection and Identification of Plant Materials

This work was carried out at the Special Research Center Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka, Anambra State.

The plant sample *Glycine max* was collected from a farmer's garden in Nibo village, in Awka South L.G.A of Anambra State (6^0 12N/, 7^0 04E/) on the 7th of June 2017. The plant specimen was identified by a herbarium curator in the Department of Botany, Nnamdi Azikiwe University, Awka, Anambra State. The voucher specimen number is NAUH-106.

2.2 Preparation of Plant Sample

The leaf and seed of *Glycine max* were dried under room temperature for a week before they were ground into a powder.

2.3 Sample Extraction

Ethanolic extract of the sample was prepared by soaking 20 g of the ground Peel in 200 ml of 70% ethanol. The mixture was placed in a shaker for one hour after which it was allowed to stand for 24 hrs at room temperature. This was then filtered using What-man paper NO. 4 and the filtrate evaporated at 80°C to dryness and the weight of the extracts noted and used to calculate the percentage yield of the extract.

The dried extract was reconstituted in 70% ethanol at a concentration of 100 ml and stored at 40°C in a refrigerator till further use.

2.4 Antioxidant Assay

2.4.1 Reducing power capacity assay

The reducing power capacity of the samples was determined using the method of [12]. This method is based on the principle of increase in the absorbance of the reaction mixture. 2.5 ml of various concentrations of ethanolic extract of the leaf and seed of *Glycine max* was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Approximately, 2.5 ml 0f 10% w/v Trichloroacetic acid was added and the mixture centrifuged at 1000 rpm for 8 min. The upper layer (5. ml) was mixed with 5. ml of deionised water followed by the addition of 1. ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. The graph of absorbance at 700 nm against the extract concentrations was plotted. Butylated Hydroxyanisole (BHA) was used as a standard antioxidant.

2.4.2 Determination of total phenol

The total phenol content of the samples was determined using the Folin-cio Caltean spectrophotometric method described by [12]. The extract solution (1 ml) was mixed with Folin and Ciocalteu's phenol reagent (1 ml). After 3 min, saturated sodium carbonate solution (1ml) was added to the mixture and adjusted to 10 ml with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (UV-Visible spectrophotometer). Gallic acid was used to make standard curve (0.01- 0.40 mM; Y = 2.8557X-0.0021; R2 = 0.9999) and the results was expressed as mg of gallic acid equivalents (GAEs) per g of extract.

2.4.3 Total flavonoids

The flavonoid content was determined by the use of a slightly modified colorimetry method described previously by [12]. A 0.5 ml aliquot of appropriately (1 mg/ ml) diluted sample solution was mixed with 2 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 min, 0.15 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, and then 2 ml of 4% NaOH solution was added to the mixture. Immediately, water was added to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15mins. Absorbance of the mixture was determined at 510 nm versus water blank with reference standard prepared with catechin concentrations. The analyses were performed in triplicate. The results were expressed as mg Catechin equivalents per 100 g of sample (mg CE/100 g).

2.4.4 β-Carotene and lycopene determination

These were determined according to the method of [12]. 0.1 g of dried ethanol extract was vigorously shaken with acetone-hexane mixture (4:6, 10 ml) for 1 min and filtered through Whatman N0. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, and 663 nm. Contents of β-carotene and lycopene were calculated using the equations:

Lycopene (g/100 ml) = -0.0458A663+ 0.372A505-0.0806A453

β-Carotene (g/100 ml) = 0.216A663- 0.304A505+0.452A453

2.4.5 Ascorbic acid determination

Ascorbic acid content of the leaf was determined using the method of [13]. 20 mg of dried leaf powder were extracted with 10 ml of 1% metaphosphoric acid. It was allowed to stand for 45 min at laboratory temperature after which it was filtered through Whatman No.4 filter paper. 1 ml of the filtrate was mixed with 9 ml of 50 µM 2, 6-dichlorophenolindophenol sodium salt hydrate and the absorbance was measured at 515 nm using a UV-Vis spectrophotometer after 30 min. Ascorbic acid content was calculated from the calibration curve of authentic L-ascorbic acid and the result expressed as mg ascorbic acid equivalent per gram of the dried sample (mgAE/g).

Qualitative phytochemical screening of the extracts was conducted to determine the presence of phytochemicals - tannins, saponins, alkaloids in addition to above flavonoid and phenol already carried out. This was done using standard procedure as described by [14].

2.5 Antimicrobial Assay

2.5.1 Test organisms

The following microorganisms (*E. coli, S. aureus*, *C. albican*, *A. niger*) were collected based on their clinical and pharmacological importance. The pure culture of microorganisms was obtained from the Department of Microbiology at Nnamdi Azikiwe University medical center, Awka, Anambra State.

2.5.2 Sample extraction

The dried extract reconstituted in 70% ethanol at a concentration of 100 ml and stored at 40°C in a refrigerator was put to use. The concentration of the extraction was determined by adding 50 g, 75 g, 100 g, and 150 g in 100 ml of ethanol. The whole set up was left for 24 hrs at room temperature and thereafter filtered using filter paper.

2.5.3 Agar well diffusion

The antimicrobial test of the plant extract was determined using agar well diffusion method as described by [15]. Both bacteria and fungi pathogens were grown first in nutrient broth before use. The pathogens were later sub-cultured in Mueller Hinton Agar. They were then bored in the Agar medium using a sterile 6 mm cork borer. The wells were then filled up with 0.02 ml of the extract and care was taken not to allow the solution to spill on the surface of the medium. The plates were allowed to stand on the laboratory bench for between 1-2 hrs to allow proper inflow of the solution into the medium before incubating the plate in incubator at 37°C for 24 hrs. The plates were later absorbed for the zone of inhibition. The effects of the extracts on bacteria and fungi pathogens were compared with those of the standard antibiotic ampicillin and fungabacter for bacteria and fungi as standard control respectively.

2.6 Statistical Analysis

The results were analyzed using ANOVA. All analyses were carried out at 5% level of significance.

3. RESULTS AND DISCUSSION

The results of the study are presented in Tables 1-5, Fig. 1 and Plates 1-2.

3.1 Antioxidant Study

3.1.1 Antioxidant phytochemical composition of the leaf and seed of *Glycine max*

The result of antioxidant phytochemical composition of the leaf and seed extracts of *Glycine max* as shown in Table 1 revealed that all the phytochemicals assayed were present but in varied amounts except ascorbic acid that was absent in the leaf. The quantities of the phytochemicals were higher in the leaf than in the seed except lycopene that was higher in the seed and ascorbic acid that was present only in the seed. The values are 1092.31±23.9 mg/100g, 877.47±19.0 mg/100g, 40.17± 0.35 g/100ml for total phenol, flavonoid and beta carotene respectively (Table 1), and 10.41± 0.16 g/100ml, 1.30±0.18 mg/g for lycopene and ascorbic acid respectively (Table 1). This result tallies with the works of these authors [16,17] who reported same in their phytochemical and antioxidant activities of *Morinda lucida* and *Talinum triangulare* respectively. The result has indicated that the leaf and seed of *Glycine max* are good sources of the phytochemicals investigated and could serve as a good antioxidant for medicinal purposes. The leaf serves a better source of total phenol, flavonoid and beta carotene while the seed serve a better source of lycopene and ascorbic acid. Plant phenolic compounds are major compounds acting as primary antioxidants by inhibiting the mutagenity of cell DNA and free radicals neutralization [18]. They are the major active ingredient in antiseptics and disinfectants due to their antimicrobial activity. Flavonoids are plant pigments without nitrogen; they are powerful antioxidants and work with carotenes to protect the plants from free radicals [18]. They possess anti-cancerous, anti-inflammatory, antimicrobial and anti-allergic activity [19] and may therefore be useful in therapeutic roles [20]. Carotenoids, present in plants and microorganisms, are mainly color pigments and contain conjugated double bonds. Their antioxidant activity arises due to the ability to delocalized unpaired electrons with resonant stabilization [21]. Carotenoids can quench singlet oxygen and react with free radicals. They can prevent damage in lipophilic compartments by scavenging peroxy radicals. Many studies have epidemiologically revealed that the consumption of diets rich in carotenoids is correlated with a

lower risk of age-related diseases [22]. Carotenoids like lycopene are found in photosynthetic pigment-protein complexes in plants, photosynthetic bacteria, fungi and algae. They are responsible for the bright orange-red colours of fruits and vegetables, perform various functions in photosynthesis, and protect photosynthetic organisms from excessive light damage. Vitamin C (ascorbic acid) is water-soluble and an important and powerful antioxidant working in aqueous environments of the body. As mentioned above, this vitamin is a partner with Vitamin E in scavenging radicals. In addition to work with vitamin E, it cooperates with carotenoids as well as with the antioxidant enzymes [23].

3.1.2 Effects of Concentration on the Reducing Power Capacity of the Leaf and Seed of *Glycine max*

In Fig. 1, the graph showed Reducing Power Capacity of the ethanol extract of the leaf and seed of *Glycine max* compared with that of Butylated Hydroxylanisole (BHA), a standard antioxidant. The concentration of the samples that produced optical density of 0.5 ($OD_{0.5}$) at 700 nm were 2.57 mg/ml and 5.15 mg/ml for the leaf and seed respectively; while that of BHA was 0.05 mg/ml. It is significant from the graph that, the *Glycine max* extracts though had a reducing power capacity but is very low in comparison with the standard used. The effectiveness of the extracts increases slightly as the concentration increases. This result is in line with that of [18] who reported that the antioxidant potentials of *Morinda lucida* leaf extract increases as the concentration increases.

3.2 Antimicrobial Study

The results in (Table 2-5) showed that *G. max* extracts at 50, 75, 100 and 150 mg/ml all had inhibitory effects on tested pathogens except at 50 mg/ml where the extracts showed inhibition only on the fungal strains (Table 3). This indicated that the plant possesses antimicrobial properties. The inhibition of bacterial strains (*S. aureus, E. coli*) suggests that the plant possesses broad spectrum of antibacterial properties which could be used in the treatment of skin diseases and food poisoning of which the pathogens are commonly implicated [8,18]. The inhibition of the fungal strain (*C. albicans* and *A. niger*) suggests also that the plant possesses antifungal property and could be used in the treatment of skin fungal infections. The antibacterial and antifungal activities of the *G. max* were linked with the presence of various bioactive compounds such as tannins, phenols, saponins, alkaloids, flavonoids, which have been found *in vitro* to have antimicrobial properties [24, 25]. From the study, the leaf showed significantly higher inhibition against the investigated microbes at all concentration when compared to the seed extract. According to [26] this could be attributed to presence of higher secondary metabolites in the leaf extract than in the seed extract. The result revealed inhibitory effect of the extracts against the microbes to be in direct proportion to the concentration of the extracts (i.e. as the concentration increases the sensitivity and susceptibility of the test organism increases). The above result is in line with the works of [27,28] who reported similar result with *Vitex chrysocarpa* and *Chromolaena odorata* respectively.

Table 1. Quantitative antioxidant phytochemical composition of the leaf and seed of *Glycine max*

Phytochemicals (mg/100 g)	Leaf	Seed
Total phenol	1092.31±23.90	938.46±4.35
Flavonoid	877.47±19.00	273.92±4.59
Beta carotene	40.17± 0.35	10.83±0.26
Lycopene	0.69±0.08	10.41±0.16
Ascorbic acid	-	1.30±0.18

Results are in mean ± standard deviation

Table 2. Inhibitory activity of the ethanol extract of *Glycine max* (leaf and seed) at 50 mg/ml concentration of the plant extract

Treatment	*S. aureus*	*E. coli*	*C. albicans*	*A. niger*
Control	12.58±0.18	9.17±0.01	$14.60±0.14^{c}$	$15.67±0.05^{c}$
Leaf	-	-	$1.79±0.01^{b}$	$2.37±0.04^{b}$
Seed	-	-	$1.16±0.00^{a}$	$1.78±0.04^{a}$
P-value	ns	ns	**	**

*Results are in mean ± standard deviation; *columns with similar alphabets are not significantly different.*
*** Significant difference exists at p<0.05, ns: Not significant; Control: Ampicillin and fungabacter for bacteria and fungi respectively*

Table 3. Inhibitory activity of the ethanol extract of *Glycine max* (leaf and seed) at 75 mg/ml concentration of the plant extract

Treatment	*S. aureus*	*E. coli*	*C. albicans*	*A. niger*
Control	15.56±0.05[b]	12.62±0.11[c]	17.87±0.01[c]	19.32±0.12[c]
Leaf	3.54±0.05[a]	3.64±0.02[b]	6.72±0.03[b]	7.63±0.04[b]
Seed	3.44±0.02[a]	2.62±0.12[a]	5.41±0.01[a]	5.71±0.13[a]
P-value	**	**	**	**

*Results are in mean ± standard deviation; *columns with similar alphabets are not significantly different at **p<0.05; ** Significant difference exists at p<0.05, ns: Not significant; Control: ampicillin and fungabacter for bacteria and fungi respectively*

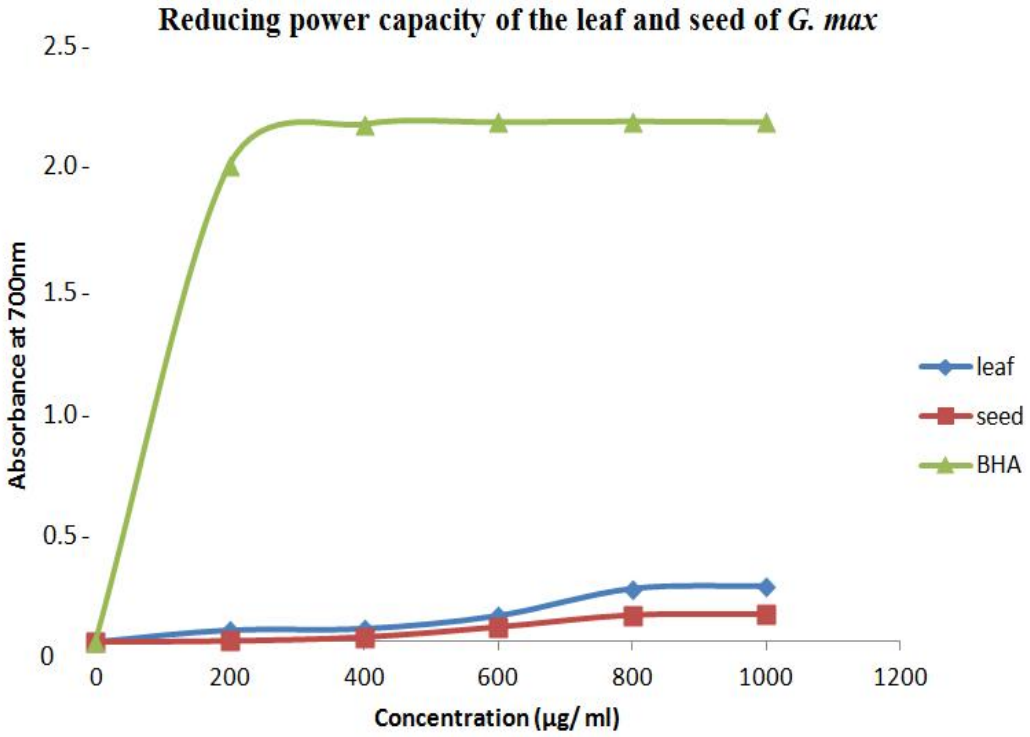

Fig. 1. Reducing Power Capacity of the ethanol extract of the leaf and seed of *Glycine max* compared with Butylated Hydroxylanisole (BHA), a standard antioxidant

Error Bars: 95% CI

Table 4. Inhibitory activity of the ethanol extract of *Glycine max* (leaf and seed) at 100 mg/ml concentration of the plant extract

Treatment	*S. aureus*	*E. coli*	*C. albicans*	*A. niger*
Control	18.30±0.14[c]	17.60±0.00[c]	21.77±0.04[c]	23.43±0.04[c]
Leaf	6.63±0.24[b]	6.32±0.05[b]	9.61±0.01[b]	10.51±0.16[b]
Seed	5.77±0.04[a]	4.66±0.05[a]	7.53±0.10[a]	7.82±0.02[a]
P-value	**	**	**	**

*Results are in mean ± standard deviation; *columns with similar alphabets are not significantly different at **p<0.05; ** Significant difference exists at p<0.05, ns: Not significant; Control: ampicillin and fungabacter for bacteria and fungi respectively*

Table 5. Inhibitory activity of the ethanol extract of *Glycine max* (leaf and seed) at 150 mg/ml concentration of the plant extract

Treatment	*S. aureus*	*E. coli*	*C. albicans*	*A. niger*
Control	21.53±0.10[c]	20.81±0.16[c]	23.63±0.24[c]	24.56±0.09[c]
Leaf	9.77±0.09[b]	9.60±0.00[b]	12.58±0.31[b]	12.43±0.04[b]
Seed	7.78±0.03[a]	6.89±0.07[a]	9.71±0.01[a]	9.29±0.01[a]
P-value	**	**	**	**

*Results are in mean ± standard deviation; *columns with similar alphabets are not significantly different at **p<0.05; ** Significant difference exists at p<0.05, ns: Not significant; Control: ampicillin and fungabacter for bacteria and fungi respectively*

Plate 1. *Glycine max* plant

Plate 2. *Glycine max* seeds

Source: Self collection

4. CONCLUSION

Data obtained from the studies showed that the plant possessed antioxidant and antimicrobial properties and could be used in the treatment of microbial infections and also to protect cells against free radical damages. However, the leaf extract showed better inhibition than the seed extract indicating that it is a better antimicrobial agent than the seed. Furthermore, the plant is an essential component of both human and animal diets and is suitable for consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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