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Free Radical Scavenging and Total Antioxidant Capacity of Combined Methanol Leaf Extract of *Solanum americanum* and *Polyalthia longifolia*

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Abstract

The present investigation highlights the phytochemical composition and free radical scavenging activity of methanol leaf extract of *Solanum americanum* and *Polyalthia longifolia*. The fresh leaves of *S. americanum* and *P. longifolia* were air-dried, milled into powder and macerated into absolute methanol. The extract was subjected to phytochemical screening and in-vitro antioxidant activity which was compared with that of standard Ascorbic acid. The qualitative quantitative phytochemical screening results constitutes flavonoids (43.05 mg/g), phenols (71.93 mg/g), alkaloids (34.11 mg/g), terpenoids (18.09mg/g), saponin (5.47 mg/g) and tannins (26.17 mg/g) all of which are known for their therapeutic properties. The antioxidant activity was evaluated using the DPPH (2, 2-diphenyl-1-picrylhydrazyl), total antioxidant capacity (TAC) and FRAP (Ferric Reducing Antioxidant Power) assays, which showed significant percentage inhibition in dose dependent manner. The combined extract exhibited significant DPPH radical scavenging activity with an Ec50 value of 0.993µg/ml, highlighting their potent radical scavenging ability. Furthermore, the FRAP assay revealed a high reducing power in the highest concentration (800ug/ml) having 79.1 µMFe^{2+}/g, suggesting effective electron donation capabilities. The total antioxidant capacity results obtained showed that the TAC for the extract was in the range of 0. 650 to 2.123 AAE (ascorbic acid equivalent) for the different concentrations used.

Keywords: Antioxidant; Ascorbic Acid; Free Radical; *Polyalthia longifolia*; *Solanum americanum*.

INTRODUCTION

Oxidation is an essential component of aerobic existence and our metabolism. During the process of oxidation, numerous free radicals are generated that possess an unpaired, newly formed electron. Atoms of oxygen or nitrogen containing central unpaired electrons are referred to as reactive oxygen or nitrogen species (Di Meo, and Venditti, 2020). This could be detrimental to the body and could lead to peroxidation of membrane lipids, aggressiveness of tissue membranes and proteins or injury to DNA and enzyme (Jomova *et al*., 2024). These might be connected to certain conditions like arthritis, heart artery conditions, cataracts, tumor, HIV/AIDS as along with age-related degenerative conditions of the brain (Mohtashami, *et al*., 2021). Currently, there is significant interest in the research of antioxidants substances primarily because of the results of the treatment impact of free radical scavengers in human health. The antioxidant activities of phenolic compounds are mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Ivanova, *et al*., 2020). In addition, they possess a metal chelating agent capability (Gulcin and Alwasel, 2022; Nwozo *et al*., 2023)

Solanum americanum, commonly known as the American black nightshade, is a versatile plant species that has garnered significant attention in the field of traditional and modern medicine. The different parts of *S. americanum* are known to be rich in a wide array of secondary metabolites, including alkaloids, flavonoids, saponins, and phenolic compounds (Patel *et al.,* 2021). In traditional medicine, *S. americanum* has been utilized for its diverse medicinal properties, with various parts of the plant being used to treat a wide range of health conditions. The leaves, for instance, have been traditionally used to treat inflammatory conditions, and anemia (Cotoraci *et al.,* 2021). The fruits and leaves have also been used to address gastrointestinal issues, such as diarrhea, and stomach ulcers (Qassadi *et al*., 2023). Beyond its medicinal applications, *S. americanum* has also been incorporated into traditional culinary practices in various regions. The plant's leaves have been consumed as a vegetable, providing a valuable source of essential nutrients and phytochemicals (Zhang *et al*.,

2023; Olaniyi *et al.,* 2024). This integration of S. *americanum* into both traditional medicine and cuisine underscores its versatility and importance in various cultural contexts.

Polyalthia longifolia, commonly known as the false ashoka is a towering evergreen tree species that has been the subject of extensive research in the field of phytochemistry and pharmacology. The different parts of this plant are known to be rich in a wide array of secondary metabolites, including alkaloids, flavonoids, and phenolic compounds (Aloke *et al.,* 2022). In traditional medicine systems, *P. longifolia* has been utilized for its diverse medicinal properties, with various parts of the plant being used to treat a wide range of health conditions. The bark, for instance, has been traditionally used to treat fever, malaria, and skin disorders (Ahmed *et al*., 2021), while the leaves and seeds have been used to address issues such as arthritis, gastrointestinal problems and to alleviate symptoms associated with neurological disorders (Jayaraman *et al.,* 2023; Iyiola *et al.,* 2024).

Several plants and plant isolates have been reported to protect free radical induced damage in various experimental models (Dutta *et al*., 2022). Previous researchers have assayed the antioxidant properties of the single extracts using different models such as Nitric Oxide, and hydrogen peroxide suggesting the antioxidant potentials of the individual extracts (Nwabiaani *et al*., 2002; Adaramola *et al*., 2017). The aim of this research is to determine qualitative and quantitative phytochemicals, in-vitro antioxidant scavenging activity of combined methanol leave extract of *Solanum americanum* and *Polyalthia longifolia*.

MATERIALS AND METHODS

Plant Materials and Preparations

Fresh leaves of *Solanum americanum and Polyalthia longifolia* used for this study were gotten and purchased from Eke-Agbani market in Nkanu West LGA, Enugu state, Nigeria. The plant samples were identified and documented by a botanist Mr Alfred Ozioko at the International Center For Ethnomedicine and Drug Development Nsukka, Nigeria. The leaves were dried in room temperature for three weeks and thereafter ground into powder with l grinder (Panasonic, Mx-AC400) and taken to the Pharmacognosy laboratory at Enugu State University of Science and Technology for extraction.

Extraction

The maceration method was employed for the extraction of the bioactive compounds from the plant materials. The fresh leaves of *Solanum americanum and Polyalthia longifolia* (600g) each were air-dried, milled into powder and macerated into absolute methanol. The mixture was agitated intermittently for 72 hours at room temperature. The resulting mixture was filtered using whatman No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure using a rotary evaporator at 40°C respectively. The extract obtained was used for phytochemical screening and evaluation of in-vitro antioxidant activity.

Phytochemical Screening

The freshly prepared extracts were qualitatively and quantitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts for tannin, phenol, flavonoids, saponin, alkaloids, terpenoids, glycosides and steroids concentrations were performed using standard procedures (Evans, 2009; Shaikh and Patil, 2020; Bakir Çilesizoğlu *et al.*, 2022). All phytochemical screening of the extracts was done in triplicate.

Measurement of free radical scavenging activity using DPPH method

Scavenging activity on DPPH free radicals by the extract was assessed according to the method reported by Gyamfi *et al.* (1999) with slight modifications. A known volume, 2.0 ml solution of the extract at different concentrations diluted two-fold (25-400 µg/ml) in methanol was mixed with 1.0 ml of 0.3 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 25 min. Blank solutions were prepared with each test sample solution (2.0 ml) and 1.0 ml of methanol while the negative control was 1.0 ml of 0.3 mM DPPH solution plus 2.0 ml of methanol. L-ascorbic acid was used as the positive control. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with UV-visible spectrophotometer. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH radical scavenging activity was calculated using the equation:

$$\%\ DPPH\ \text{Inhibition}\ = 100\ \% \times \left(\frac{\mathrm{A_0} - \mathrm{A_s}}{\mathrm{A_0}}\right)$$

A_o is the absorbance of the control while A_s is the absorbance of the test sample. All free radical scavenging activity assays were done in triplicate.

Total Antioxidant Capacity

Total antioxidant capacity of the extracts was determined using the phosphomolybdate method as described by Saeed *et al* (2012) using Ascorbic acid as a standard drug. An aliquot of 0.1 ml of each of the extract and ascorbic acid at varying concentrations (10-320 µg/ml) were mixed with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped with aluminium foil and incubated in a water bath at 95 °C for 90 minutes. After the samples had cooled at room temperature, the absorbance of each mixture was

measured at a wavelength of 765 nm against a blank. A typical blank contained 1 ml of the reagent solution and incubated under the same conditions. All tests were performed in triplicate. The antioxidant capacity was estimated using Ascorbic acid equivalent (AAE)

Ferric Reducing Antioxidant Power

The ferric-reducing antioxidant power of the extract was determined by the method of Benzie and Strain (1999). Different concentrations (25 – 800µg/mL) of the extract (0.5 ml) were mixed with 0.5 ml phosphate buffer (pH 6.6) and 0.5 ml 0.1 % potassium hexacyanoferrate, followed by incubation at 50°C in a water bath for 20 minutes. After incubation, 0.5 ml of 10 % TCA was added to terminate the reaction. The upper portion of the solution (1 ml) was mixed with 1 ml of distilled water and 0.1 ml of 0.01 % $FeCl_3$ solution. The reaction mixture was left for 10 minutes at room temperature and the absorbance was measured at 700 nm against appropriate blank solution. All tests were performed in triplicate. A higher absorbance of the reaction mixture indicated greater reducing power. Gallic acid was used as a positive control. Then, iron colour reagent (0.05 ml) was added to all the test tubes and mixed. The tubes were incubated in a water bath at 37°C for 10 minutes. Thereafter, the absorbance of all the test tubes was measured at a wavelength of 700 nm.

Statistical Analysis

The experiments were carried out in triplicate and results are given as the mean ± standard deviation. Statistical analysis was carried out using one way ANOVA with Duncan post hoc test.

RESULTS

Preliminary phytochemical screening of the combined leave extract of *S. americanum and P. longifolia* revealed the presence of various bioactive components of which flavonoids and Phenol were the most prominent and the results of phytochemical tests are summarized in Tables 1and 2

DPPH Radical Scavenging Assay

The scavenging ability of the combined extracts of *S. americanum and P.longifolia* assay is shown in Table 3. The percentage of inhibitions (57.4, 61.5, 75.6, 86.6, 93.8 %) was increasedwith increasing concentrations of the extract. The EC50 value for DPPH scavenging activity of the extract was found to be 0.993µg/ml.

Total Antioxidant Capacity (TAC)

The total antioxidant capacity (TAC) was based on the reduction of phosphate-molybdenum (VI) to phosphate-molybdenum (V) by the combined extracts and subsequent formation of green phosphate/ Mo (V) complex at acidic pH. It evaluates both water-soluble and fat-soluble antioxidants.The results obtained showed that the total antioxidant capacity for the extract was in the range of 0. 650±0.01 to 2.12±0.00AAE (Ascorbic Acid Equivalent) as shown in Table 4.

Ferric Reducing Antioxidant Power (FRAP)

As shown in Table 5, the combined methanol leaf extract of *S. americanum* and *P. longifolia* leaves had significantly ($p<0.05$) higher reducing capability in all the concentrations used for FRAP investigation. This is shown in the number of Fe^{3+}ions reduced to Fe^{2+} in $\mu MFe^{2+}/g$, which was concentration-dependent. The reducing ability of the extracts (42.1, 46.1, 52.1, 59.2, 73.3, 79.1 $\mu MFe^{2+}/g$) was found to be significant ($p<0.05$) in all concentrations. A higher absorbance of the reaction mixture indicated greater reducing power

Table 1. The results of preliminary phytochemical screening.

PhytochemicalsConstituents	Bioavailability
Tannin	++
Phenol	+++
Flavonoids	++
Saponin	+
Alkaloids	+++
Terpenoids	++
Glycosides	+
Steroids	ND

Key: *+low concentration, ++ Moderate concentration, +++ High concentration, ND Not Detected*

Table 2. The results of quantitative phytochemical screening.

Bioactive Compounds	Combined Methanol Extract Quantity (mg/g) (Mean ± SD)
Flavonoids	43.05 ± 2.2
Tannins	26.17 ±1.2
Saponin	5.47 ± 3.5
Alkaloids	34.11±4.8
Phenols	71.93±1.4
Terpenoids	18.09 ±2.9
Glycosides	10.03 ±1.1
Steroids	13.11±01

Table 3. DPPH scavenging activity on combined *Solanum americanum and Polyalthia longifolia* extract.

Concentration (µg/mL)	% inhibition Combined extract	% inhibition Ascorbic acid
25	57.4	53.3
50	61.5	61.6
100	75.6	72.8
200	86.6	80.0
400	93.8	93.9
EC_{50}	0.993	

Table 4. Total antioxidant capacity on combined *Solanum americanum and Polyalthia longifolia*extract.

Concentration µg/mL	Mean ±SD TAC (AAE)
10	0.650±0.01
20	0.793±0.03
40	0.863±0.05
80	0.990±0.03
160	1.753±0.11
320	2.123±0.02

Total antioxidant capacity of combined extract of S. americanum and P. longifolia. Data are presented as mean value ± standard deviation SD (n = 3)

Table 5. Ferric reducing power capacity (FRAP) on combined *Solanum americanum and Polyalthia longifolia* extract.

Concentration (µg/mL)	Combined methanol ExtractsµMFe^{2+}/g
25	42.1±0.01
50	46.1±2.3
100	52.1±0.02
200	59.2±1.00
400	73.3±0.01
800	79.1±0.02

Ferric reducing antioxidant power of combined extract of S. americanum and P. longifolia. Data are presented as mean value ± standard deviation SD (n = 3)

DISCUSSION

S. americanum and *P. longifolia* are commonly used traditionally to treat many diseases whose pathogenesis are, among other factors, linked to reactive oxygen and nitrogen species (Behl *et al*., 2021). However, information on antioxidant potentials of the combined plants that could be beneficial in the treatment and management of such diseases has not been investigated. In this study, we report the antioxidant potentials of combined leaf extract of *S. americanum* and *P. longifolia.* Phytochemical screening of methanol extract of *S. americanum and P. longifolia* showed the presence of flavonoids (43.05 mg/g), phenols (71.93 mg/g), alkaloids (34.11 mg/g), terpenoids (18.09 mg/g), saponin (5.47 mg/g) and tannins (26.17 mg/g). Phytochemicals are currently receiving increased attention due to interesting new findings on their biological activities. The phytochemicals detected in this extract might be involved in the therapeutic action of this plant part. The high phenolics and flavonoids content of this extract indicate high antioxidant potentials because the phenolics constituents can react with active oxygen radicals such as hydroxyl radical, superoxide anion radical and lipid peroxy radical (Gulcin, 2020; Tumilaar *et al*., 2024).

Free radicals are powerful pleiotropic distruptors of physiological function like smooth muscle relaxation, neuronal communication, prevention of platelet aggregation, and control of cell-mediated toxicity (Sahebnasagh *et al*., 2022). Certain free radicals such as nitric oxide serve multiple roles as effector molecules in various biological systems, functioning as neuronal messengers, promoting vasodilation, and exhibiting antimicrobial and anti-inflammatory effects (Belenichev et al., 2024). Various literature reports showed that there is high correlation between antioxidant activity and phenolics content (Sotiropoulou *et al*., 2020; Martínez *et al*., 2022). The presence of these biological active compounds suggests that the plant could serve as a potential source of drugs and its secondary metabolites could exert some biological activities when taken by humans. The reaction with the free radical is widely taken as a common mechanism of lipid peroxidation. Radical scavengers may directly react with and quench peroxide radicals to end the peroxidation chain reactions that are crucial in pathogenesis of various diseases (Engwa *et al*., 2022).

Test Based on the use of DPPH radicals is one of the most popular spectrophotometric methods for determination of Antioxidant capacity of plant and plant based extracts because radical compounds can react directly with antioxidants. Current results indicate that extract is apparently good free radicals and probably have the ability to inhibit lipid oxidation and may be beneficial in the treatment of various diseases where lipid peroxidation is important mechanism for pathogenesis.

The total antioxidant capacity (TAC) was based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of green phosphate/Mo(V) complex at acid pH. It evaluates both water-soluble and fat-soluble antioxidants (total antioxidant capacity). The results indicate higher TAC (expressed as ascorbic acid equivalent) of the methanol extract at a low concentration. It was, however, observed that the extract possesses significant total antioxidant capacity at higher concentration (Table 4). The strong antioxidant capacity might be associated with the presence of the secondary metabolites. Previous studies recorded that phenolic compound including flavonoids are associated with strong antioxidant activity and possess healthy benefits ((Sotiropoulou *et al*., 2020; Ofoedu *et al*., 2021).

As shown in table 5, combined *S. americanum and P. longifolia* leave extract exhibited increased ferric reducing power with increased concentration. In ferric reducing antioxidant power assay, the presence of antioxidants in the extract would result in reducing Fe^{3+} to Fe^{2+} by donating an electron by the extract. The extract with reducing power showed that they are electron donors, reduce the oxidized intermediates and act as primary antioxidant substances (Pisoschi *et al*., 2021). In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of each compound present in the leave extract. The compounds with reduction potential react with potassium ferricyanide (Fe^{3+}) to form potassium ferrocyanides (Fe^{2+}), which then react with ferric chloride to form ferric ferrous complex that is

greenish in colour. The amount of Fe^{2+} complex was monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increasing absorbance at 700 nm indicated an increase in reductive ability. The higher absorbance of extract may be due to its strong reduction potential and the presence of the bioactive compounds in the extract which possess potent electron donating abilities. This assay further confirmed the antioxidant properties of the extracts observed from the DPPH assay. The FRAP values obtained in this study was similar to the results reported by Vijayalakshmi, and Ruckmani, 2016 who recorded similar result for n-butanol extract of *Anchomanes difformis*. The correlation between reducing power and DPPH values could be due to the same mechanism on which these methods rely. The results clearly indicated that the extract examined can act as electron donors and react with free radicals, and convert them to more stable products, thus terminating the radical chain reaction and preventing pathological diseases.

CONCLUSION

Phytochemical screening of this extract revealed the presence of several secondary metabolites with known biological antioxidant activities. The extract also showed a strong antioxidant activity by scavenging DPPH and FRAPS methods. In addition, the extract was found to contain relatively high levels of total phenolics and flavonoids, which play a major role in controlling oxidation generated by free radicals.

Conflict of Interest: The authors declare that they have no conflict of interests.

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