Phytochemical Evaluation and Pharmacological Activities of Leaf and Fruit Extracts of African Blackberry Nightshade - *Solanum Nigrum* Linn. *(*Niprd H 7357)

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Abstract. Solanum nigrum is a tropical plant sometimes used as a vegetable for consumption in some communities where they grow in the wild, with anecdotal reports of its usefulness as a medicinal plant, especially in treating asthma. This present study aimed to determine the phytochemical constituents of the leaf, fruit, and seed of *Solanum nigrum* Linn. extracts and evaluate its pharmacological activities via ATBS and DPPH assays evaluate its pharmacological activities of the chloroform, ethyl acetate, and methanol extracts.

The researcher collected fresh leaves and fruits of privately cultivated *Solanum nigrum* Linn. from FCT Abuja, Nigeria, extracted them using solvents (chloroform, ethyl acetate, and methanol), and evaluated the pharmacological activities of the various extracts via ATBS and DPPH assays.

Alkaloids, tannins, phenolics, flavonoids, steroids, flavosterols, fixed oils, terpenoids, and saponins were present in both the leaf and fruit extracts of *Solanum nigrum*. The fruits, however, have higher concentrations of phenols, flavonoids, and vitamins B1, B2, and C. Our Study further showed that the ethyl acetate and methanolic extracts exhibited high ABTS and DPPH scavenging capacity. The high ABTS and DPPH scavenging capacity by the ethyl acetate and methanolic extracts suggests that the plant has immunomodulatory, anti-inflammatory, hepatoprotective, and antioxidant properties and authenticate its traditional use in the treatment of asthma. Therefore, future research on the effect of plant extracts on smooth muscles and, subsequently, clinical trials on asthma patients is recommended.

Keywords: *Solanum nigrum* Linn.; phytochemicals; ABTS; DPPH; scavenging; asthma.

INTRODUCTION

Solanum nigrum Linn. (also known as African blackberry nightshade, garden nightshade, hierba mora, garden huckleberry, wonder berry, hound's berry, and petty morel) is a common edible herb of the Solanaceae family, which is widely distributed in subtropical and tropical regions of Europe, Asia, America, Africa, and Australia [1-4]. Natural extracts have been used for treating human diseases for more than 2000 years and are becoming popular in this modern era, mainly due to their low cost, availability in rural areas, low toxicity and profound therapeutic benefits compared to synthetic drugs [5]. A plant is considered to have therapeutic potential if any of its parts has been shown through rigorous research to have beneficial effects for treating an illness [6]. Indeed, many potent drugs have been obtained from plants, including artemisinin, quinine, and emesis and have been introduced into

modern clinical practice. Over 80% of the world population relies on plant-derived agents for preserving health and treating illnesses [7].

Solanum nigrum Linn. is utilised as a vegetable and fruit in many parts of the world; its stem, leaves and seeds are used for soup preparation in parts of the world where it is cultivated [1, 8]. The herb has been extensively used in folk medicine [8, 9]. Though the effect of *Solanum nigrum* Linn. on bronchial smooth muscle is unknown, its antioxidant, anti-inflammatory and immunomodulatory properties suggest that it can be used in asthma management. Unfortunately, there is little justification to substantiate this herb's use in asthma management [1].

Figure 1 – African Blackberry Nightshade (*Solanum nigrum* NIPRD H 7357)

African blackberry nightshade or *Solanum nigrum* is a common plant in Nigeria. It is called Igi Odan in Yoruba and is used as an herb for treating asthma in the locals. Thus, researchers suggest using *Solanum nigrum* extract as an adjuvant in managing asthmatic patients. Though the effects of *Solanum nigrum* Linn. on bronchial smooth muscle are unknown, the antioxidant, anti-inflammatory, antihistaminic, antiallergic, and immunomodulatory properties of this herb suggest it can manage asthma. Indeed, β-sitosterol, a bioactive molecule identified in *Solanum nigrum*, has been demonstrated to act as an anti-asthmatic agent. Experts recommend integrating herbal medicines with health care programs. However, the lack or inadequacy of scientific data on herbal medicines has substantially affected the widespread acceptance of these plant-derived agents [6, 7].

This present study aimed to determine the phytochemical constituents of the leaf, fruit, and seed of *Solanum nigrum* Linn. extracts and evaluate their pharmacological activities via ATBS and DPPH assays.

METHODS

Collection, Identification and Preparation of Plant Materials. Fresh wild grape (*Solanum nigrum* Linn.) plant samples were collected from Abuja, FCT Nigeria, where they were privately cultivated on the residential premises of AB under standard conditions with adequate access to sunlight and aeration. The plant was authenticated at the National Institute for Pharmaceutical Research and Development (NIPRD) FCT Abuja Nigeria, with a voucher number NIPRD H 7357 allotted. Leaves and seeds of the plant were subsequently collected, gently washed and air-dried in the Medical Biochemistry Laboratory of the College of Health Sciences, Nile University, FCT Abuja, Nigeria. The plant materials were blended using a mechanical blender while the leaves and fruit powder were collected into zip-lock bags and stored until use.

Extraction of Plant Materials. The resulting leaf powder (60 g each) was weighed and soaked in 100 ml of methanol, ethyl acetate and chloroform in 3 different covered glass jars and placed on an orbital shaker maintained at 300 rpm for 48 hours, thus, extracted via maceration. The resulting extracts were each filtered using Whatman No1 filter paper, and the filtrates were placed on a rotary evaporator and subsequently concentrated to dryness in a water bath at 40 °C. These yielded Methanolic Leaf Extract of *Solanum nigrum* Linn. (MLSN), Ethyl Acetate Leaf Extract of *Solanum nigrum* Linn. (EALSN) and Chloroform Leaf Extract of *Solanum nigrum* Linn. (CLSN). The same extraction drying and concentration to dryness procedures were carried out on the fruit powder to give Methanolic fruit Extract of *Solanum nigrum* Linn. (MFSN) and Ethyl acetate fruit Extract of *Solanum nigrum Linn.* (EAFSN). All the extracts were subsequently stored in the refrigerator at (-4˚C) until use.

Chemicals and Reagents. DPPH, 2, 4-dinitrophenyl hydrazine (DNPH), Folin Ciocalteu's phenol reagent, quercetin, and hydrogen peroxide were procured from Sigma Aldrich, St-Louis USA. Methanol, ethyl acetate, and n-hexane were obtained from Scharlab S.L., Gato Perez, 33-

P. I. Mas d'En Cisa. Other chemicals and reagents were of analytical grade and prepared in laboratory glassware with distilled water.

Phytochemical Screening. Each extract of *Solanum nigrum* Linn. (MLSN, EALSN, CLSN, MFSN and EAFSN) was screened for its secondary metabolite constituents according to the standard methods [10, 11] with slight modifications as follows:

Qualitative Determination of Secondary Metabolites. Alkaloids. To 1 ml of 1% HCl, 3 ml of each extract of *Solanum nigrum* Linn. was added and heated for 20 minutes, cooled, and filtered. The researcher added a few drops of Wagner's reagent (2 g of iodine and 6g of KI in 100 ml of distilled water) to 1 ml of the filtrate. A reddish-brown precipitate indicated the presence of alkaloids.

Tannins. The researcher added 1 ml of each Solanum nigrum Linn extract to 1 ml of freshly prepared 10% KOH. A dirty white precipitate indicated the presence of tannins.

Phenolics. The researcher added 1 ml of each Solanum nigrum Linn extract to 2 drops of 5% $FeCl_3$ nanoparticle. A greenish precipitate indicated the presence of phenolics.

Glycosides. To 10 ml of 50% H_2SO_4, 1 ml of each extract of *Solanum nigrum* Linn. was added and heated in boiling water for 15 minutes, and 10 ml of Fehling's solution was added and cooked again. A brick-red precipitate indicated the presence of glycosides.

Saponins. The researcher added 3 ml of each Solanum nigrum Linn extract to 5 drops of olive oil in a test tube and shook it vigorously. A stable emulsion indicated the presence of saponins.

Flavonoids. A researcher added 3 ml of the extract to 1 ml of 10% NaOH. A yellow colouration indicated the presence of flavonoids.

Steroids. A researcher added 1 ml of each Solanum nigrum Linn extract to 5 drops of concentrated H_2SO_4 nanoparticle. A red colouration indicated the presence of steroids.

Phlobatannins. A researcher added a few drops of 1% HCl to 3 ml of each extract of *Solanum nigrum* Linn. A red precipitate indicated the presence of phlorotannins.

Triterpenes. A researcher added five drops of acetic anhydride to 1 ml of each Solanum nigrum Linn extract, followed by a drop of concentrated H_2SO_4 surveyed. The researcher steamed the mixture for 1 hour, neutralised it with NaOH, and added chloroform. A blue-green colour indicated the presence of triterpenes.

Quantitative Determination of Total Phenolics, Total Flavonoids and Vitamins B and C. Secondary metabolites and vitamins were quantitatively determined using previously described methods:

Total Phenolics (Folin-Ciocalteu's Method). The researchers added 0.5 ml of *Solanum nigrum* Linn. extract to 10 ml of deionised distilled water and 2.5 ml of 0.2 N Folin-Ciocalteu's phenol reagent. They left the mixture undisturbed at room temperature for 5 minutes and added 2 ml of 2% sodium carbonate. The absorbance of the resulting solution was read at 780 nm and repeated three times. Quercetin served as a standard for the calibration curve [10–11].

Total Flavonoids. The researchers added 1.5 ml of each *Solanum nigrum* Linn. extract to 1.5 ml of 2% methanolic $AlCl_3$ solution. They vigorously shook the mixture on an orbital shaker at 200 rpm for 5 minutes and measured the absorbance at 367 nm after incubating it for 10 minutes. They used quercetin as a standard for the calibration curve and generated results in triplicates [12].

Riboflavin (Vitamin B2). The researchers conducted the assay as previously described [13], with slight modifications. They extracted a known amount (5 g) of the sample with 100 ml of 50% ethanol solution and shook it for 1 hour. After filtering the mixture into a 100 ml flask, they pipetted 10 ml of the extract into a 50 ml volumetric flask, added 10 ml of 5% potassium permanganate and 10 ml of 30% H_2O_2, and allowed it to stand over a hot water bath for about 30 minutes. Then, they added 2 ml of 40% sodium sulfate, increased the volume to 50 ml, and measured the absorbance at 510 nm. They performed this assay in triplicate.

Ascorbic Acid (Vitamin C). The researchers determined this as previously described [14]. They thoroughly mixed 0.5 ml of each *Solanum nigrum* Linn. extract with 1.5 ml of 6% TCA and then centrifuged at 3500 g for 10 minutes. After centrifugation, they mixed 0.5 ml of the supernatant with 0.5 ml of DNPH reagent and allowed it to stand at room temperature for 3 hours. Then, they added 2.5 ml of 85% tetra-oxo-sulphate (VI) acid and left it undisturbed for 30 minutes before measuring the absorbance at 530 nm. They also processed a set of standards containing 10–50 μg of

ascorbic acid and a blank. The researchers performed this assay in triplicate.

2,2-diphenyl-1-picrylhydrazyl DPPH Radical Scavenging Capacity. The ability of *Solanum nigrum* Linn. extracts to bleach the purple colour of 2,2-diphenyl-1-picrylhydrazyl or DPPH radical was determined as described by the authors [15]. The researchers added 2 ml of various concentrations (0.2–1.0 mg/ml) of each extract to 2 ml of 0.1 mmol/l methanolic DPPH solution. After incubating the mixture in the dark at room temperature for 30 minutes, they measured the absorbance against the control at 517 nm. They then calculated the scavenging rate (I%) on the DPPH radical using the given expression:

$$I\% = [(A\ control - A\ sample) / A\ control)] \times 100$$

where A control is the absorbance of the control reaction (containing all reagents except the test compound); A sample is the absorbance of the test compound. The procedure was carried out in triplicate.

Ferric ion Reducing Antioxidant power. The ability of *Solanum nigrum* Linn. extracts to reduce Fe^{3+} to Fe^{2+} was evaluated by slightly modifying the previously described procedure [16]. Briefly, various concentrations of the extracts (0.2-1.0 mg/ml) were suspended in 1 ml of distilled water, mixed with 250 µl of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K3Fe (CN)6]. The researchers incubated the mixture at 50°C for 20 minutes, then added 250 µl of 10% trichloroacetic acid and centrifuged at 4000 rpm for 10 minutes. They mixed 250 µl of the supernatant with an equal amount of distilled water and 0.5 ml of 0.1% $FeCl_3$. Finally, they measured the absorbance of the resulting solution at 700 nm. They performed the procedure in triplicate.

2,2′-Azino-bis radical ABTS Radical Cation Decolorization Assay. The decolourisation of 2,2′-Azino-bis radical cation ($ABTS^{\bullet+}$) was measured as previously described [15] with minor modifications. $ABTS^{\bullet+}$ solution was prepared by mixing aqueous ABTS (7 mM) solution with 2.45 mM potassium persulfate (1:1 v/v) and incubating in darkness at room temperature for 16 hours. The researchers prepared the working solution by diluting the $ABTS^{\bullet+}$ solution in methanol until it reached an absorbance of 0.70 ± 0.05 at 734 nm. They added 25 µl of varying concentrations of the plant sample to 200 µl of the working solution in each well of a 96-well plate. After gently shaking the plate, they covered it with aluminium foil and kept it at room temperature for 30 minutes. Finally, they recorded the absorbance. The following formula calculated the ABTS radical decolorising activity.

$$\text{ABTS radical decolorising activity (\%)} = (1 - A\ sample/A\ control) \times 100.$$

RESULTS AND DISCUSSIONS

The use of medicinal plants for their pharmacological advantages has been documented since time immemorial [5, 7]. This study identified alkaloids, tannins, phenolics, flavonoids, steroids, flavosterols, fixed oils, terpenoids and saponins as constituent metabolites with saponins present only in the methanol extracts of the leaves, as well as vitamins B1, B2 and C in the fruits and leaves of *Solanum nigrum* – Tables 1, 2.

Table 1 – Phytochemical constituents of *Solanum nigrum* NIPRD H 7357

Metabolites	CLSN	MLSN	EALSN	MFSN	EAFSN
Alkaloids	+	+	+	-	+
Tannins	+	+	+	+	+
Phenolics	+	+	+	-	+
Glycosides	-	-	-	-	-
Saponins	-	+	-	-	-
Flavanoids	+	+	+	+	+
Steroids	+	+	+	+	+
Phlobatamines	-	-	-	-	-
Triterpenes					
Phytosterols	+	+	+	+	+
Fixed Oils	+	-	-	+	+
Terpenoids	+	+	+	+	+
Amino acids	-	-	-	-	-

Notes: + = present, - = absent, MLSN = Methanolic Leaf Extract of *Solanum nigrum* Linn., EALSN = Ethyl Acetate Leaf Extract of *Solanum nigrum* Linn., CLSN = Chloroform Leaf Extract of *Solanum nigrum* Linn., MFSN = Methanolic Fruit Extract of *Solanum nigrum* Linn. and EAFSN = Ethyl acetate Fruit Extract of *Solanum nigrum* Linn.

The ethyl acetate extract of the fruit showed significantly higher concentrations of total phenol and flavonoid when compared to the ethyl acetate extract of the leaves.

Table 2 – Total Phenolics, Flavonoids and Vitamins B and C in Various extracts of *Solarium nigrum*

Solvent	Chloroform Extract		Ethyl acetate Extract		Methanolic Extract	
	Fruit	Leaf	Fruit	Leaf	Fruit	Leaf
Total Phenol (mg/ml Quercitin)	48.92 ± 0.59**	15.20 ± 4.24##	88.77 ± 0.94*	14.62 ± 3.71###	20.52 ± 3.24***	42.59 ± 2.51#
Total Flavanoids (mg/ ml Quercitin)	65.58** ± 1.15	40.44 ± 0.37###	80.65 ± 1.27*	49.96 ± 0.35#	36.44 ± 2.56***	42.38 ± 0.49##
Vitamin B_1 (mg/dL)	15.88 ± 2.65#	0.43 ± 0.03	35.31 ± 1.97*	0.55 ± 0.07	22.68 ± 3.05**	0.75 ± 0.11
Vitamin B_2 (mg/dL)	0.17± 0.04#	ND	0.381 ± 0.01*	ND	0.229 ± 0.01**	ND
Vitamin C (mg/dL)	87.74 ± 5.60#	ND	256.34 ± 12.77*	ND	127.35 ± 17.52**	ND

Notes: The results are expressed as Mean ± Standard error of the Mean for triplicate determinations in various extracts. Values with the same superscript are not significantly different.

Likewise, the chloroform extract of the fruit also showed significantly higher concentrations of total flavonoids and phenols when compared to the leaves. Both extracts had relatively higher amounts of total flavonoids and phenols when compared to the methanolic extract. There was a significantly lower vitamin B1 content in the leaf extracts compared to the fruit extract, even as vitamins B2 and C, present in the fruits, were not detected in the leaf extracts – Table 2.

The major bioactive compounds are the steroidal saponins, constituting approximately 40%, and steroidal alkaloids, approximately 13% [2, 12]; this is consistent with previous documentation that *Solanum nigrum* has at least 188 phytochemicals obtained from the leaves, whole plant, stems, roots, fruit, and seeds. The phytochemicals include steroidal saponins, steroidal alkaloids, terpenes, lignans, sterols, phenolic compounds, coumarins, organic acids, flavonoids, phenylpropanoids, proteins, carbohydrates, lipids [1, 5, 17, 18] and volatile oils, among others [2]. However, the major bioactive compounds are the steroidal saponins, constituting approximately 40%, and steroidal alkaloids, approximately 13% [2, 12].

From our study, the ethyl acetate extract of the fruit showed higher FRAP-reducing activity at higher concentrations when compared to the other extracts, followed by the chloroform and methanolic extracts, respectively, when compared to other extracts. Moreover, ascorbic acid and butylated hydroxytoluene extracts of African blackberry nightshade had the highest ferric-reducing antioxidant power (Figure 2).

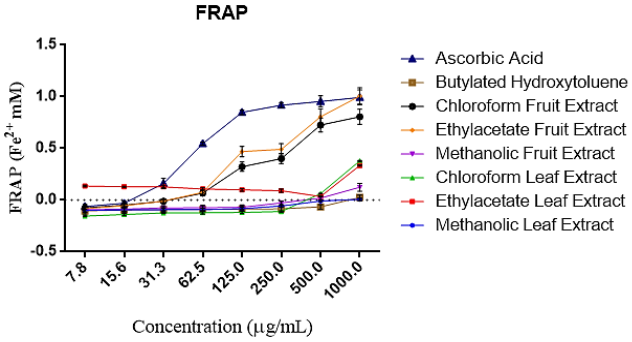

Figure 2 – Ferric reducing antioxidant power (FRAP) of various extracts of *Solanum nigrum* Linn.

Notes: The values expressing Mean ± Standard error of mean triplicate determinations from various concentrations.

Ascorbic acid and butylated hydroxytoluine extracts of African blackberry nightshade had the highest 2,2-diphenyl-1-picrylhydrazyl (or DPPH) scavenging capacity. The chloroform leaf extracts closely followed these (Figure 3).

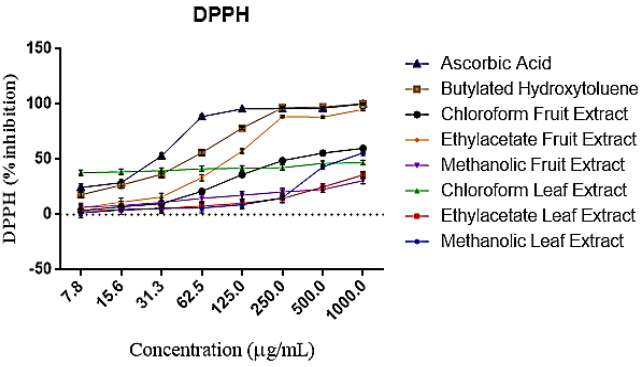

Figure 3 – 2,2-diphenyl-1-picrylhydrazyl or DPPH scavenging capacity of various extracts of *Solanum nigrum* Linn.

All the extracts had good ABTS (2,2′-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) scavenging capacity at high concentrations. However, ascorbic acid and butylated hydroxytoluine extracts were more effective than those at low concentrations (Figure 4).

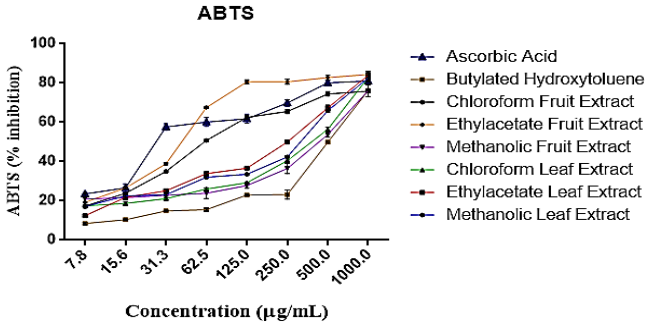

Figure 4 – ABTS (2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid) scavenging capacity of various extracts of *Solanum nigrum* Linn.

Notes: The values are expressed as mean ± Standard error of mean triplicate determinations from various concentrations.

Our present work also showed that the ethyl acetate extract of the fruit showed significantly higher concentrations of total phenol and flavonoid when compared to the ethyl acetate extract of the leaves. Likewise, we detected vitamins B1, B2 and C, but there was a significantly lower vitamin B1 content in the leaf extracts compared to the fruit extract. The steroidal alkaloids identified in the literature include β2-solasonine, lignin amides, nicotinic acid, cannabis in F, adenosine, adenine, 9-amino nonane-1, 3, 9-tricarboxylic acid, allantoin, solar acid, solamargine, solasonine, the glycoside of solamargine and solasonine, tigogenin, spirosestanol glycoside, and furostanol glycosides. These alkaloids possess anti-inflammatory properties and exhibit a potent inhibitory activity against several types of cancers [1, 2, 4, 12, 19]. The 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical cation-based assays are among the most abundant antioxidant capacity assays, together with the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-based assays according to the Scopus citation rates [20]. According to this present study, the ABTS assay revealed that EAFSN and EALSN have the most scavenging antioxidant capacity owing to their high inhibition rate, followed by those of ascorbic acid and butylated hydroxytoluene. Furthermore, the DPPH assay revealed that EAFSN and MLSN have the most antioxidant capacity owing to their high inhibition rate compared to the other extracts. Several phenolic compounds, including gallic acid, catechin, protocatechuic acid, chlorogenic acid, gentisic acid, caffeic acid, luteolin, apigenin, epicatechin, rutin, naringenin scopoletin, salicylic acid, vanillic acid, 2, 4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, and 2, 5-dihydroxybenzoic acid have been identified in *Solanum nigrum* extract. The leaves of *Solanum nigrum* are particularly rich in polyphenols, including phenolic acids and flavones. These compounds have various pharmacological activities such as antioxidant, anti-inflammatory, antibacterial, and antiviral [1, 2, 19].

The *Solanum nigrum* carbohydrates are mainly polysaccharides, but disaccharides have also been discovered [19–20]. The biological effects of polysaccharides are due to their immunomodulatory, anti-inflammatory, antitumor, hepatoprotective, and antioxidant properties [2, 19].

CONCLUSIONS

The high ABTS and DPPH scavenging capacity by the ethyl acetate and methanolic extracts suggests that the plant has immunomodulatory, anti-inflammatory, hepatoprotective, and antioxidant properties and, as such, authenticates its traditional use in treating asthma. Therefore, future research on the effect of plant extracts on smooth muscles and subsequent clinical trials on asthma patients is recommended.

Conflict of Interest

Authors hereby declare no conflict of interest.

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