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QUANTITATIVE PHYTOCHEMICAL ANALYSIS AND THE ANTIOXIDANT PROPERTIES OF THE METHANOL EXTRACT FROM THE LEAVES OF *Solanum americanum.*

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ABSTRACT

This study estimated the quantity of some phyto- compounds as well as the antioxidants activities of the methanol leave extract of *Solanum americanum* [S.a]. The quantitative phytochemical analysis were carried out using gravimetric and spectroscopic method while the antioxidant activities were assayed using free radical scavenging activities of DPPH, Nitric oxide, hydrogen peroxide and ABTS. The quantitative analysis showed that the phenolic content of the leaves to have highest concentration at 62mg/100g alkaloids 60mg/100g, total flavonoids and tannins 48 and 45mg/100g respectively. The extract showed inhibitory potential against DPPH free radical, the inhibitory percentages vary from 33.68±0.74 to 80.83±1.10 at 100µg/ml for the methanol extract of the plant *Solanum americanum* to 48.19±0.73 to 84.59±0.91 at 100µg/ml for the standard [ascorbic acid]. The maximum NO scavenging activity of the plant extract to ascorbic acid are 77.89±0.69 to 89.19±0.35 at a concentration of 100µg/ml. The IC_{50} value are 47.79 for the inhibition of the plant extract while that of the standard is 27.80. ABTS assay free radical scavenging percent inhibition has an increased concentration from 25-100 µg/ml at 33.75±0.87-71.92±0.15. H_2O_2 radicals scavenging activity inhibition percentage of methanol leaf extract of the plant *Solanum americanum* was from 53.82±0.37 to 76.89±0.32 at 25-100µg/ml as compared to the standard with inhibition percentage from 78.35±0.79 to 95.18±0.37. The quantitative analysis showed that the phenolic content has the highest concentration, hence has the most antioxidant activity also that the plant has strong free radical scavenging activities.

Key words: Phytochemicals, methanolic extract, Phenolic content, Flavonoid, Tannin, *Solanum Americanum.*

INTRODUCTION.

The plant *Solanum americanum* [*S.a.*] belongs to genus solanum and the family Solanaceae. This family is renowned for their pharmacological activities in the body system of humans when consumed in appropriate quantities. *Solanum americanum* [American black nightshade] is a perennial herb. This plant is found in some African countries like Kenya, Cameroun, Sierra Leone, Tanzania, Ghana, Madagascar, Mauritius, South Africa, and Nigeria. [1-2]. It grows in swampy areas or on the shore. Seeds are produced

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in plentiful quantities in plants sprouting between the months of April through July.[3].

The purpose of this study is to investigate free radical scavenging and estimation of the antioxidant properties of methanol extract of the plant *Solanum americanum. Solanum americanum* is consumed in most West African homes as herb and leafy vegetable. In recent times, antioxidants available in food nutrients have captured the interest of scientists. Scientist have come to the realization that plant phytochemicals and nutrient conscious feeding could combat the unwholesome cases of tumor growth, hypertension, diabetes and untimely aging. [4]. Medical scientists are of the opinion that consistent intake of phytochemicals [antioxidants] present in plants may improve the accumulation of appropriate antioxidants in the body for its normal functioning. [5].

MATERIALS AND METHODS

Materials:

Samples of leaves of *Solanum americanum* were harvested in March 5th 2020 from the swampy ground of Akesan Village in Igando, Alimosho local government area of Lagos state. Identification of the plant as *Solanum americanum* was done by Dr. O.K. Oluwa of the department of Botanv, Lagos state university, Ojo local government area. Lagos state. Voucher number Nwabiani 001-005. Analytical grade reagents and double distilled water were purchased from MC Donald Scientific emporium.

Laboratory Procedure.

Extraction of plant parts with methanol:

The leaves of the plant samples were shade dried and powdered using a grinder. The powdered sample was stored in desiccator. 10g of powdered sample was weighed, put in conical flask, 100 ml of methanol was added and plugged with cotton. This was allowed for one day at room temperature with continuous stirring. The supernatant was filtered and the solvent was evaporated by cold maceration method to get the crude extract. The filtration was done with Whatmann No.1 filter paper. The crude extract was stored in airtight bottles in a refrigerator for subsequent usage. [6].

Phytochemical screening.

Estimation of total tannin content:

The test for the total tannin content was done following the method described by [7]. The tannins were determined by Folin-Ciocalteu method.

Total phenolic content* [*TPC*]*:

The total soluble phenolic content was done by the method of using the Folin -Ciocalteu‘s phenol reagent [8], with tannic acid as standard.

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Reducing sugar content:

Standard glucose solution was made according to the method employed by [9] with slight modification [10 mg glucose/100ml]. Sugar content was determined using the Nelson – Somogyi method.[10-11]

Total Alkaloid Content Determination:

The total alkaloid was carried out according to the method employed by [12]**.**

Estimation of Total Flavonoid Content:

The estimation of total Flavonoid content was done by the methods described by [13].

Antioxidant Assays.
DPPH in vitro free radical scavenging activity:

The 1, 1-diphenyl-2-picrylhydrazyl [DPPH] radical scavenging method as described by [6] was used to evaluate the antioxidant property with slight modification. The plant's antioxidant properties were compared to ascorbic acid. 1.5 ml of 0.1 mM DPPH solution was mixed with 1.5 ml of various concentrations [25 to 100 µg/ml] of leaf extract. The mixture was shaken vigorously and incubated at room temperature for 30 min in the dark. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm by a spectrophotometer. The solution without any extract and with DPPH and methanol was used as control. The antioxidant activity of each sample was expressed in terms of IC_{50}, and was calculated from the graph after plotting inhibition percentage against extract concentration. This test was done in triplicates.

Nitric oxide radical scavenging activity*:*

Nitric oxide radical scavenging activity of *Solanum americanum's* extracts was measured by the method described by [14]. Different concentration of test sample [25, 50, 75, 100µg ml^{-1}] in methanol.

Radical scavenging activity of Hydrogen peroxide:

The ability of the extract to scavenge hydrogen peroxide was determined in accordance with modified method given by [15].

ABTS•+[2,2'-azino- bis[3-ethyl benzothiazoline -6- sulfonate] radical cation ASSAY PROTOCOL:

ABTS•+ radical cation decolorisation assay was done following the method of [16] with slight modification. The oxidant is produced by persulfate oxidation of 2, 2-azinobis [3-ethylbenzoline-6-sulphonic acid] [$ABTS^{2-}$]. ABTS radical cation [$ABTS^{\cdot+}$] was gotten by reacting 10ml ABTS solution [7mM] with 10ml of 2.45mM potassium persulphate solution and the mixture were allowed to stand in dark room temperature for 12-16 hours before use. For this study different concentrations of [25-100µg/ml] of methanolic extract [0.5ml] were added to 0.3ml of ABTS solution and the final volume was made up with ethanol to make 1 ml. The

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absorbance was read at 745nm. Solvent blanks were run in each assay for accurate readings.

RESULTS AND DISCUSSION.

The mean of three absorbance at various concentration [25- 100 µg/ml] of *S.a.* as well as standard Ascorbic acid [25 -100 µg/ml] were calculated and plotted using Microsoft excel 2010 and GraphPad Prism 6 software respectively. The results of antioxidant activities are shown below: The mechanisms involved in antioxidant activity assay is the capacity of a molecule of a compound to donate a hydrogen atom to a radical, and the readiness for the hydrogen donation is the most valuable factor involved in free radical scavenging [17]. The results of the DPPH assay showed that the inhibitory percentages vary from 33.68±0.74 to 80.83±1.10 for the methanol extract of the plant *S.a.* to 48.19±0.73 to 84.59±0.91 for the ascorbic acid. This showed a cardinal increase in the scavenging activity with the increase in concentration. Highest antioxidant activity was given in the concentrations of 75µg/ml and 100µg/ml in the plant's extract as compared to the standard with the same concentration. However, the extract of *S.a.* tested against DPPH stable radicals spectrophotometrically revealed that the radical scavenging activity of *S.a.* methanol extract possessed excellent antioxidant capacity by increased inhibition with the increasing concentration of the extract. The dose response curve of DPPH radical scavenging activity of crude extracts of plant was observed, when compared with standard ascorbic acid and shown in figure 1. Thus it is obvious that polyphenolic antioxidants in the leave of the plant *Solanum americanum* has an essential bioactive property and the DPPH scavenging effect can be associated with the presence of active phytoconstituents in the leaves of the plant. DPPH antioxidant inhibition values were increased in dose dependent manner. This shows that the plant has strong antioxidant properties. Plant acts as electron donors because of the possession of phenolic constituents in their bioactive compounds [18] this may support DPPH radical scavenging activity reported in the extracts tested. This further explains why the DPPH scavenging properties of plant extracts increase with the concentration of extracts [19-20].

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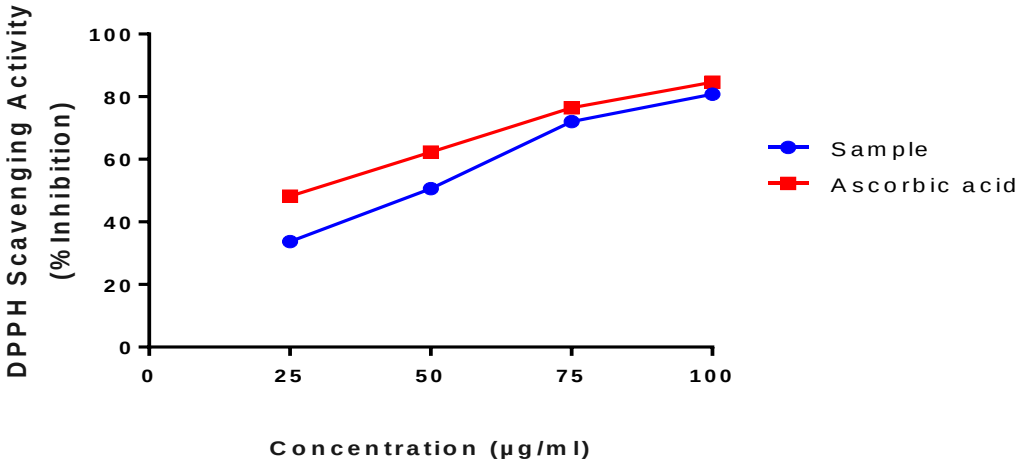

Figure 1: DPPH scavenging activity of *S. a.* in comparison with ascorbic acid.

Table 1: % DPPH Scavenging activity [% Inhibition] of *S. a./ascorbic acid*

Conc µg/ml	Percentage inhibition	
	S. a.	*ascorbic acid*
25	33.68±0.74	48.19±0.73
50	50.65±0.55	62.31±0.91
75	72.02±0.74	76.43±0.73
100	80.83±1.10	84.59±0.91
IC_{50}	42.52	28.22

n = 3. Values are given in mean ± SD.

The methanol extract recorded maximum percentage of NO scavenging activity of 77.89% at the concentration 100 µg/ml and 89.19% at the concentration 100 µg/ml figure 2 and table 2 show the percent NO scavenging activity of the extract and the standard. Nitric oxide [NO] is an essential chemical mediator produced in the endothelial cells, macrophages, neurons, etc. It is the major player in the control of different physiological processes [21]. Ialenti et al reported that too much concentration of NO in the body can lead to several diseases [22]. It is observed that *Solanum americanum* is less than ascorbic acid in its inhibition of NO. The maximum NO scavenging activity of the plant extract to ascorbic acid are 77.89±0.69 to 89.19±0.35 at a concentration of 100µg/ml. The IC_{50} value are 47.79 for the inhibition of the plant extract while that of the standard is 27.80. Phenolic and flavonoids compounds have been reported to act

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as antioxidative compounds in biological systems, playing the part of scavengers of singlet oxygen and free radicals [23]. The result of this research work suggest that the plant has the antioxidant property to fight the effect of NO production. This is because of the presence of alkaloids, steroids, tannins, saponins, flavonoids, flavonones and phenols. These antioxidants are reasonably responsible in stopping the detrimental effects of excessive NO generation in the body. Antioxidant potential of *Solanum americanum* methanol extract was estimated by using potassium ferric cyanide reduction method. The yellow colour of the test solution changes to different shades of green and blue. By measuring the formation of Pearl's Prussian blue at 700 nm using a spectrophotometer, it is possible to determine the concentration of ferrous ions. Absorbance increase shows the plant's abundant reductive properties. [24].

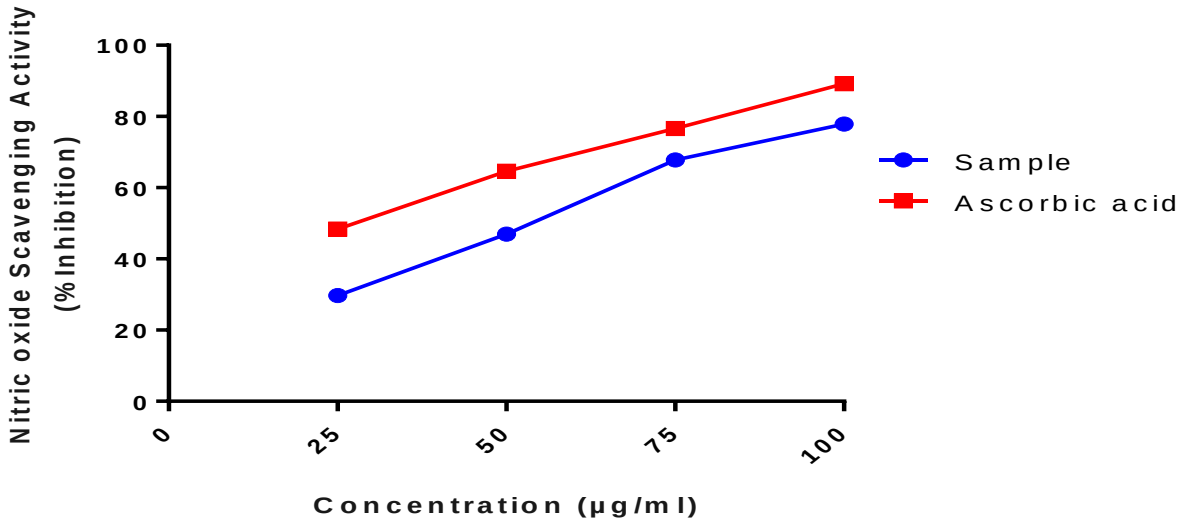

Figure 2: Nitric oxide scavenging assay of methanolic extract of *Solanum americanum* compared to the standard.

Table 2: % Nitric oxide Scavenging activity [% Inhibition] of *S.a /ascorbic acid.*

Conc. µg/ml	Percentage inhibition	
	S. a.	*ascorbic acid*
25	29.73±1.05	48.41±0.35
50	46.93±0.35	64.62±0.69
75	67.81±0.69	76.66±0.69
100	77.89±0.69	89.19±0.35
IC_{50}	47.79	27.80

n = 3. Values are given in mean ± SD

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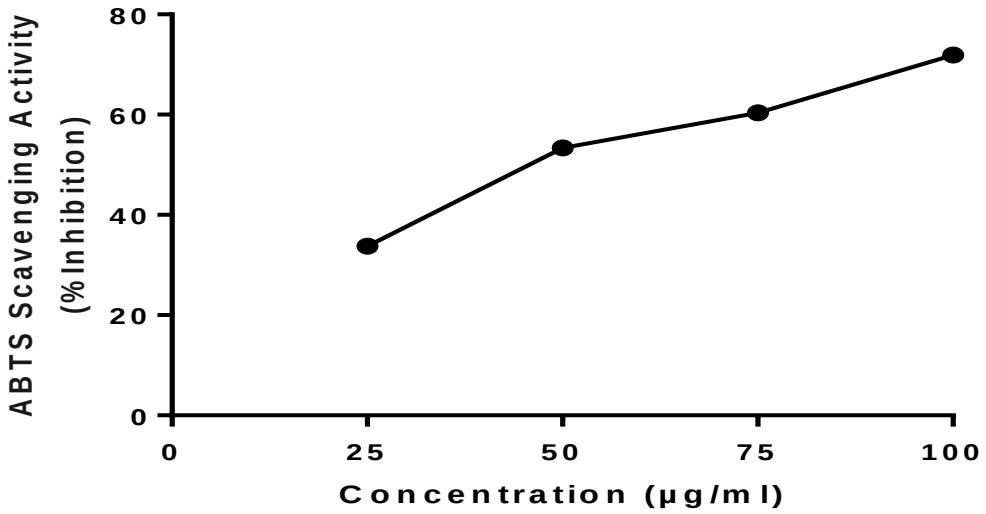

Figure 3: ABTS scavenging activities of methanolic extract of *Solanum americanum.*

Table 3: ABTS scavenging Activity [%Inhibition] of *Solanum americanum.*

Concentration	Percentage inhibition
25µg/ml	33.75±0.87
50µg/ml	53.40±0.15
75µg/ml	60.39±0.44
100µg/ml	71.92±0.15
IC_{50}	46.04

n = 3. Values are given in mean ± SD

Solanum americanum's methanolic leave extract showed increased ABTS assay free radical scavenging inhibition with increasing concentration from 25-100 µg/ml at 33.75±0.87-71.92±0.15. IC_{50} ABTS scavenging Activity [%Inhibition] of *Solanum americanum* was recorded as 46.04.

The result showed that the extract had strong H_2O_2 scavenging activity which may be due to the presence of antioxidant compounds. Antioxidant constituents present in the extracts are good electron donors which may hasten the production of H_2O from H_2O_2. Figure 4 reports the H_2O_2 scavenging activity of various concentrations of leaf extract of the plant *Solanum americanum* as compared to the standard. The *Solanum americanum* has shown an encouraging H_2O_2 radicals scavenging activity with a good inhibition percentage of 53.82±0.37 to

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76.89±0.32 from 25-100 µg/ml as compared to the standard with inhibition percentage from 78.35±0.79 to 95.18±0.37 at the same concentration . The IC_{50} values of the leaf extract of the plant *Solanum americanum* is 20.38% as compared to standard ascorbic acid of 7.85%. This shows that the leaf extract of the plant *Solanum americanum* has a moderate inhibition percentage as compared to the standard. The hydroxyl radical is a reactive free radical produced in biological system. These are implicated with the responsibility of destroying almost every molecule found in living system thereby causing lipid peroxidation and biological harm. [25-26]. The Hydroxyl radical scavenging inhibition percent of the plant *Solanum americanum* was found between 25-100µg/ml concentration. compared to other phytochemicals that were evaluated. Total phytochemical content evaluation showed that phenolic content is 63.78±0.33mg. It also showed that the plant has more percentage of phenolic compounds.

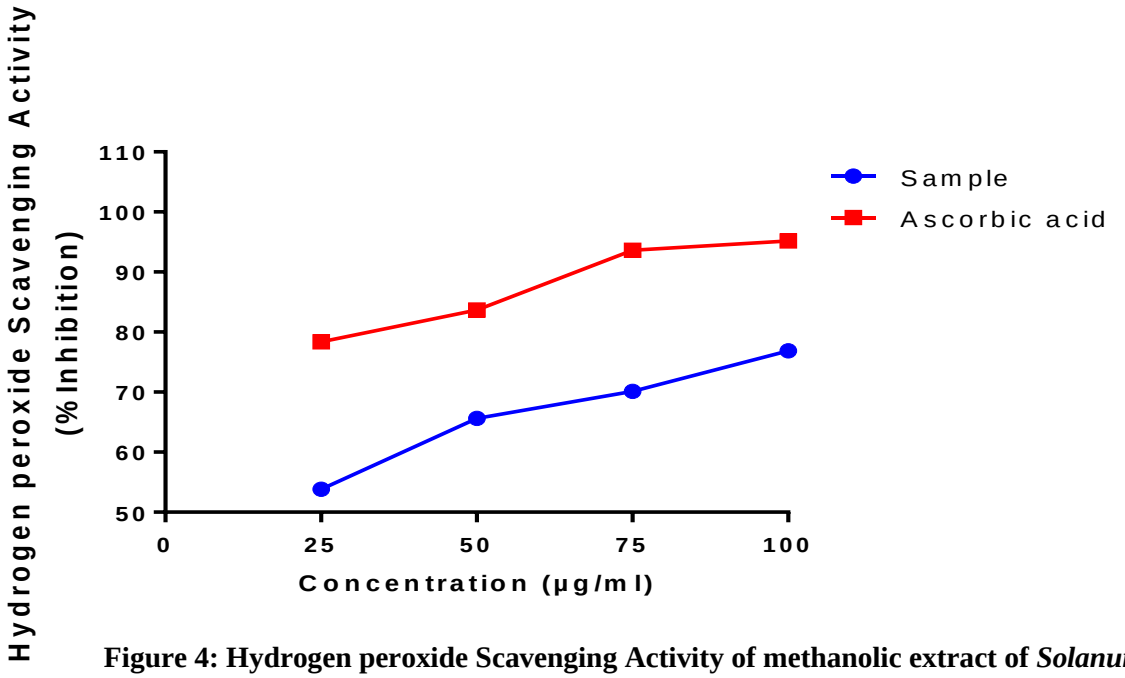

Figure 4: Hydrogen peroxide Scavenging Activity of methanolic extract of *Solanum americanum* compared to the standard.

Table 4: Hydrogen peroxide Scavenging Activity of *S.a /ascorbic acid.*

Conc µg/ml	Percentage inhibition	
	S. a.	*ascorbic acid*
25	53.82±0.37	78.35±0.79
50	65.63±0.79	83.70±0.74
75	70.12±0.91	93.65±0.32

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100	76.89±0.32	95.18±0.37
IC_{50}	20.38	7.85

n = 3. Values are given in mean ± SD

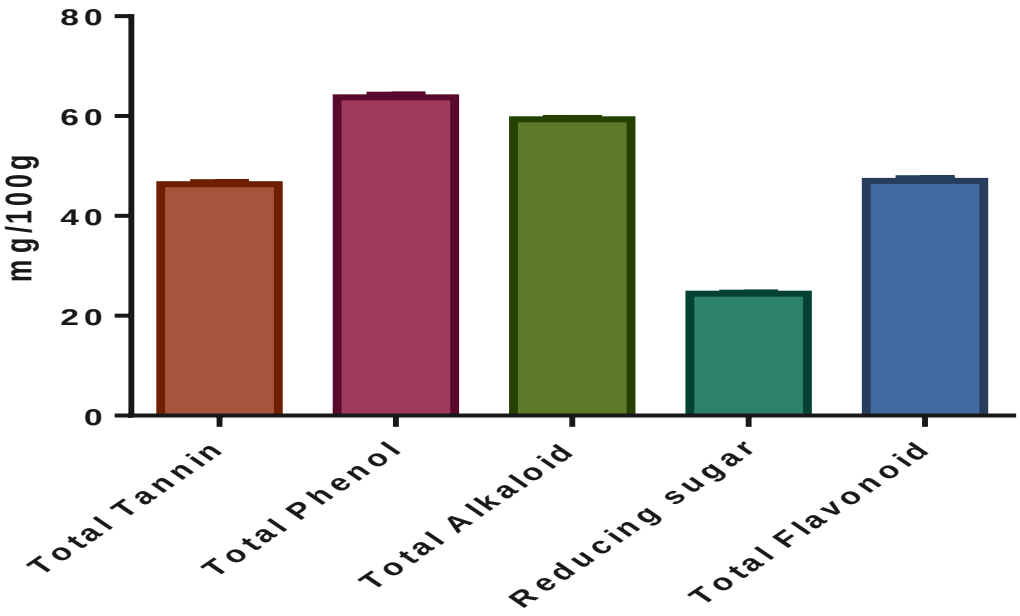

Figure 5: Total antioxidant tests of the leaves of *Solanum americanum*

QUANTITATIVE TESTS.

Table 5: Total antioxidant tests of the leaves of *Solanum americanum*

Total Tannin mg/100g	46.40 ±0.40
Total Phenol mg/100g	63.78 ±0.33
Total Alkaloid mg/100g	59.34 ±0.31
Reducing sugar	24.41±0.20
Total Flavonoid mg/100g	47.00±0.58

n = 3. Values are given in mean ± SD

CONCLUSION: The present investigation demonstrates the

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presence of total phenolic, flavonoid, reducing sugar, alkaloid and tannin content of the leaf extract of the plant *Solanum americanum.* The free radical-scavenging properties of *Solanum americanum* extract could be as a result of plentiful presence these phytochemicals. The results obtained from the antioxidant activity of this plant leaf extract showed that the extracts possess strong antioxidants which contain high level of phenolic content. The antioxidant ability of plant phenolic compounds is because they react as reducing agents, hydrogen donators, singlet oxygen quenchers and metal chelators. [27]. Hence, the presence of a plentiful amount of these phytochemicals is a strong suggestive lead that this plant could have medicinal properties.

REFERENCES.

1. E. A. Olet, M. Heun, and K. A. Lye. [2005]. African crop or poisonous nightshade;the enigma of poisonous or edible black nightshade. *African J. of Ecology,* 43[2], 158-161.

2. D.E. Symon, [1981]. *A revision of the genus solanum in Australia.* Illustrated edition, volume 4,Australia. Adelaide Botanic Gardens, pp. 1-367.

3. P.Keeley and R. J. Thullen,[1983]. Influence of planting date on the growth of black nightshade Solanum nigrum. *A.J. of Weed Science,* 31[2], 180-184.

4. M.F. Delany, C.T. Moore, D.R.P. Rogulske, [1993]. Survival and longevity of adult male Florida Grasshopper Sparrows. Proceedings of the annual conference of the south eastern association of fish and wildlife agencies, 47, 366-9.

5. A.K.Goyal, T.Mishra, M.Bhattacharya, P. Kar and A. Sen [2013]. Evaluation of phytochemical constituents and antioxidant activity of selected actinorhizal fruits growing in the forests of Northeast. *Indian J. of Bioscience,* 38[4], 797-803.

6. S.Anjali, and S.Sheetal, [2013]. Phytochemical Analysis and Free Radical Scavenging Potential of Herbal and Medicinal Plant Extracts. *Indian J. of Pharma. and Phytochemistry.* 2[4], 22-29.

7. M.Govindappa, S.Naga Sravya, M.N.Poojashri, T.S.Sadananda, and C.P. Chandrappa,[2016]. In Kavitha Chandran C.I. and Indira G. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of Strobilanthes Kunthiana [Neelakurinji].*Indian J. of Med. Plants Studies,* 4[4], 282-286.

8. V.L. Singleton, and J.A. Rossi, [1965]. Colorimetry of total phenolics with phosphomolybdic phosphor tungstic acid reagents. *Am. J. of EnologyViticulture,* 16, 144-158.

9. Y.H. Pratiwi, O. Ratnayani, I.N. Wirajana,[2018]. Comparison of Reducing Sugar Test Methods in Determining LArabino furano sidase Activity With Coconut [Cocos Nucifera] Leaves Substrate, J. Kim.; 134-139.

10. H.K. Al-kayyis and H. Susanti, [2016]. Comparison of Somogyi-Nelson and Anthrone-Sulfate Methods for Determination of Reducing Sugar Content in Cilembu [Ipomea batatas L. Bulbs]. J. Pharm. Sci. Community. 13[2] 81–89.

11. N.Endang, P. S. Yuliana, D. Elsi, Sumardi , Q.I.Hardoko and W. Sri,

ISSN 0795 - 22066

[2022]. Analysis of reducing sugar levels of Cattleya sp. orchid plantlet after induction fusaric acid in vitro. W. J.of Advanced Research and Reviews. 14[02], 095–099.

12. M. Jayaramu, R. M. Prabhavathi, and M. P.Prasad [2016]. Studies on Qualitative and Quantitative Phytochemical Analysis of Cissus quadrangularis. *Egyptian J. of Advances in Applied Science Research*, 7[4], 11-1.

13. C.I Kavitha Chandran. And G. Indira [2016]. Quantitative estimation of total phenolic, flavonoids, tannin and chlorophyll content of leaves of Strobilanthes Kunthiana [Neelakurinji]. *Indian J. of M. Plants Studies*. 4[4], 282-286

14. L.C.Green, D.A.Wagner, J. Glogowski, P.L.Skipper, J.S. Wishnok and S.R.Tannenbaum, [1982]. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. *Am. J. of Analytical Biochemistry*, 126[1], 131-138.

15. R.J.Ruch, S. Cheng and J.E. Klaunig,[1989]. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *J. of Carcinogenesis*, 10[6], 10031008.

16. R. Re, N. Pellegrini, A. Protegent, A. Pannale, M.Yang and C.Rice-Evans, [1999]. Antioxidant activity applying an improved ABTS radical cation assay. *J. of Free Radical Bio. and Med.*, 26[9-10], 1231–1237.

17. G.Miliauskas, P.R.Venskutonis and T.A.van Beek, [2004]. Food Chem. 85, 231

18. S. Duthie and V.Dobson,[1999] Dietary flavonoids protect human colonocyte DNA from oxidative attack in vitro. Eur J Nutr. 38:28–34.

19. M Gul, F. Ahmad, A, Kondapi I.Qureshi and I. Ghazi [2013]. Antioxidant and antiproliferative activities of Abrusprecatorius leaf extracts - an in vitro study. BMC Complement Alternat Med. 13[53].

20. Y .Hilmi, M .Abushama, H. Abdalgadir, A. Khalid and H. Khalid,[2014]. A study of antioxidant activity, enzymatic inhibition and in vitro toxicity of selected traditional sudanese plants with antidiabetic potential. BMC Complement Alternat Med 14[149].

21. H. Lata and G.K. Ahuja, [2003]. Role of free radicals in health and disease. Ind. J. Physiol. Allied Sci.57: 124-28

22. S. Ialenti, M. Moncada and Di Rosa, [1993]. Modulation of adjuvant arthritis by endogenous nitric oxide.Br. J. Pharmacol. 110: 701-705

23. L.V. Jorgensen, H.L.Madsen, M.K. Thomsen, La Dragsted and L.H. Skibsted, [1999]. Regulation of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy. Free Rad. Res. 30: 207- 220.

24. R.Tauheed, A. A.Muhammad, Aziz-ur-Rehman, S .Tayyaba, A. Muhammad and M.K. Khalid,[2012]. Phytochemical screening, free radical scavenging,antioxidant activity and phenolic content of Dodonaea viscose Jacq. Journal of Serbian Chemical Society. 77[4];423-435.

25. H. Kappus, O.Aruoma, and B.Halliwell,[1991] Lipid peroxidation; Mechanism and biological relevance. Free radicals and food additives, London: Taylor and Francis Ltd. pp.59-75.

26. T. Fossen and O. M. Andersen, [2003]. Anthocyanins from red onion Allium cepa with novelaglycone.

ISSN 0795 - 22066

Phytochemistry J. 62: 1217–1220.

27. N.C.Cook and S. Samman, [1996]. Flavonoids-chemistry, metabolism, cardioprotective effects, and dietary sources. J. Nutr. Biochem., 7:66-76.

ISSN 0795 - 22066