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Research Article

Antioxidant Activity of Senna Alata

Preliminary Quantitative Phytochemical, Physicochemical And *In-Vitro* Antioxidant Activity Of *Senna Alata* Hydroalcoholic Leaf Extract

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Abstract : Many oxidative stress related diseases are as a result of accumulation of free radicals in the body. Present studies are directed towards finding natural antioxidants of plants origins. The present study was carried out to determine the phytochemical constituents, its qualities and antioxidant activity of *Senna alata* hydroalcoholic leaf extract as per the standard pharmacopoeial protocol. The aim of this study was to evaluate *in vitro* antioxidant activities and to screen for phytochemical constituents of *Senna alata*. The free radical scavenging activity of hydroalcoholic extract against various assay in terms of IC_{50} value were DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay (715.26µg/ml), reducing power assay (715.26µg/ml), ABTS test (643.95µg/m) and Nitric oxide test (856.79µg / ml) was evaluated. The phytochemical analysis revealed the presence of secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, anthroquinone, phenols, saponin, tannin and carbohydrates. Quantitative phytochemical analysis revealed the amount of Alkaloid content (49.2%), Flavonoid content (6.96%) and Phenolic content (69.72%) present. Also the Physicochemical examination analysis were also carried out in *Senna alata* powdered sample which showed the presence of Total Ash (10.25 ± 0.04), Acid Insoluble Ash (2.75 ± 0.03), Water Soluble Ash (1.98 ± 0.02), Alcohol Extract Value (6.32 ± 0.03), Water Extract Value (11.19 ± 0.02), Loss on Dry (6.14 ± 0.03) and Foreign Matter (0.38 ± 0.01). Our findings provide evidence that the crude hydroalcoholic extract of *S.alata* is a potential source of natural antioxidants, and this justified its uses in folkloric medicines.

Keywords: Qualitative Phytochemical Analysis, Quantitative Phytochemical Analysis, Physicochemical Analysis, Antioxidant Activity, *Senna Alata.* Hydroalcholic Extract

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1. INTRODUCTION

It's a paradise of regenerating thoughts that plants deliver to humanity, and it's been serving as the best source of phytochemicals since the dawn of time[1]. A large number of new pharmaceuticals were banned from floristic assets, while others were used based on their use in standard medicinal treatments. For a long time, a variety of restorative plants have been used in everyday life to combat ailments all over the world[1]. The common practise of using home-grown treatments in medical care arrangements, such as those depicted in old writings known as 'ethno-drugs,' was adopted as therapeutic items[1]. India's medicinal plant collection is regarded as vast. The largest vault with a large number of therapeutic and sweet-smelling plants is India's backwoods, which is mostly used as raw material for the creation of drugs and related goods. Ayurveda is gaining popularity and recognition as the world's most ancient medical system. The World Health Organization (WHO) has classified 21,000 plants used for medicinal reasons all around the world. India is the world's largest supplier of therapeutic spices, with a diverse range of agro-climatic conditions, and is renowned as the world's professional flowerbed[2]. Phytoconstituents are the bioactive combinations found in plants on a regular basis. These phytoconstituents operate in conjunction with supplements and other components to treat a variety of ailments [3]. Phytochemicals are generally divided into two categories: essential and auxiliary elements. The essential constituents include starches, amino acids, proteins, and chlorophyll, whereas the auxiliary constituents are alkaloids, terpenoids, steroids, and flavonoids [3]. Alkaloids have a role in metabolism and aid in the improvement of living conditions. It is utilised as an antioxidant, anti-provocative, and to lower the risk of cancer in smokers. Tannin possesses antiviral, antibacterial, and antiparasitic properties, as well as antiulcer properties[5]. Flavonoids and phenolic chemicals found in plants have been shown to have a variety of biological effects, including cell strengthening, and anti-carcinogenic properties [6]. A few elements such as sodium, potassium, magnesium and calcium are needed in large quantities by the body are called large-scale components[7]. Micro components are metals such as chromium, manganese, iron, zinc, nickel, and copper that are required in small amounts [4]. The presence of metals like cadmium, mercury, and lead in food and drugs, which are harmful to one's health, is regulated and monitored by the country's legal authorities. This last category is known as significant metals [8]. Plants are a rich source of chromium, which is required for glucose digestion; iron, which is a component of haemoglobin; zinc, which is required for cell division, protein synthesis, and insulin digestion; nickel, which is required for active beta-cells in the pancreas [9]; and copper, which is a component of ceruloplasmin, is required for haemoglobin formation [10]. Oxidation is a key step in the metabolic cycle in living organisms, involving both responsive (hydrogen peroxide) and non-receptive (hydroxyl revolutionary (OH) groups [11]. Rapid free radical improvement can alter the design and capability of cell parts and surfaces, resulting in human neurological and other issues such as malignant growth, diabetes, provocative illnesses, asthma, cardiovascular diseases, neurodegenerative infections, and premature maturation [12]. To avoid the aforementioned situations, cancer prevention medications or free radicals should be readily available in the body for free radical search [13]. Nitric oxide and free radicle peroxy nitrite, like superoxide anions, hydrogen peroxide, and hydroxyl, play a vital role in microorganisms assessing several major oxidation linked illnesses[14]. The most effective way to eliminate and reduce the activity of free radicals that generate oxidative stress is to utilise cell supplementation assurance systems [15,16]. Cell reinforcements are compounds that weaken the features of the free extreme chain response. Potentially useful medicinal herbs have recently sparked renewed interest in reducing tissue injury caused by oxidative stress as cancer prevention agents cell reinforcements [17,18]. In many non-industrial countries, *S. alata* (L.) Roxb, a delicate wood shrub known as winged *Senna*, ringworm bush or stomach gizzard plant, forest lord or flame cassia, is a crucial therapeutic plant. Skin sicknesses and various skin ailments are treated with the concentrates from the leaves alone or mixed with lime juice, or vegetable oil [19, 20]. In Ghana, the approach is to rub the affected area by the *S.alata* plant oil blend onto the bruises. The current study was a preliminary phytochemical analysis and *in-vitro* cell study of *S.alata* hydroalcoholic concentrate

2. MATERIALS AND METHODS

2.1 Collection and Extraction of Plant material

S. alata leaves were collected and the voucher specimen was kept in the Alpha Omega Hi-Tech Bio research focus (Voucher No: AORF125). With running tap or potable water, the plant materials were cleaned and shade dried. The leaves and barks were crushed to powder. In 250 ml of Chloroform, ethyl acetic acid, ethanol, methanol, and hydro alcohol, and these coarse powders (25 g) were then dispersed for continuous extraction using Soxhlet apparatus. The concentrates were collected and then subjected for further evaluation.

2.2 Phytochemical Screening

Preliminary phytochemical analysis was carried out for all extracts of *S. alata* as per standard methods[21,22].

2.3 Quantitative Phytochemical Analysis

2.3.1 Determination of Alkaloids

Alkaloid was determined by Harborne strategy[23]. About 5g of the hydroalcoholic extract of *S. alata* test was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added, covered and permitted allowed to settle for4 hrs. This was then separated and the concentrate was focused on a water shower to one-fourth of the first volume. Concentrated ammonium hydroxide was added drop astute to the concentrate until the precipitation was finished. The entire arrangement was permitted to settle and the encouraged was gathered and washed with weakened ammonium hydroxide and then transferred. The collected sample is the alkaloid, which was dried and gauged.

2.3.2 Determination of Flavonoids

Ten grams of hydroalcoholic extract of *S. alata* test was repeatedly separated with 100ml of 80% aqueous methanol at room temperature. The combination was then transferred through a filter paper into a pre-weighed 250ml beaker. The filtrate was transferred into a water bath and

allowed to dryness and weighed. The quantity of flavonoid was determined by difference in weight[24].

2.3.3 Determination of Total phenols

The fat-free sample was taken in beaker with 50 ml of ether for the extraction of the phenolic part for 15 min. 5 ml of the concentrate was pipetted into a 50 ml conical flask, then, at the point 10 ml of refined water was added. 2 ml of ammonium hydroxide arrangement and 5 ml of concentrated amyl liquor were additionally added. The hydroalcoholic extract of *S. alata* tests was left to respond for 30 min for shading advancement. This was estimated at 505 nm in spectrophotometer[25].

2.3.4 Physico-chemical analysis

The physicochemical analysis was done according to the standard method for the determination of extractive value, total ash content, acid insoluble ash, water-soluble ash and loss on drying percentage[26].

2.4 Antioxidant activity

2.4.1 DPPH Radical Scavenging Activity

Molyneux Method[27] completed the DPPH radical scavenging assay. To 1.0 ml of 100.0 µM DPPH sample taken and diluted in methanol, a similar volume of hydroalcoholic leaf of *S. alata* test in various concentrations were added and kept for 30 minutes in room temperature. The change in coloration was observed in terms of absorbance using a spectrophotometer at 514 nm. 1.0 ml of methanol was added to the control tube rather than the test.

2.5 Reducing Power assay

The sample together with Ascorbic acid solutions were spiked with 2.5ml of phosphate buffer (0.2 M, pH 6.6) and 2.5ml of 1% potassium ferricyanide. The mixture was kept in a 50°C water-bath for 20min. The resulting solution was cooled rapidly, spiked with 2.5ml of 10% trichloroacetic acid, and centrifuged at 3000rpm for 10 min. The supernatant (5ml) was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride and incubated for 10min. The absorbance was detected at 700nm on spectrophotometer. The extract concentration providing the absorbance was calculated from the graph of absorbance at 700 nm against extract concentration. Ascorbic acid was used as standard. Higher absorbance indicates higher reducing power[28].

2.6 ABTS radical scavenging activity

ABTS radical scavenging assay of the hydroalcoholic leaf extract in *S. alata* not really set in stone[29]. The ABTS +cation radical was created by the response between 5 ml of 14 mM ABTS arrangement and 5 ml of 4.9 mM potassium persulfate ($K_2S_2O_8$) solution, stored in the dark at room temperature for 16 hrs. Before use, this solution was diluted with ethanol to get an absorbance of 0.700 ± 0.020 at 734 nm. The plant extract at various concentrations with 1ml of ABTS solution was homogenized and its absorbance was recorded at 734 nm. Ethanol blanks were run in each assay, and all measurements were done after at least 6 min. Similarly, the reaction mixture of standard group was obtained by mixing 950 µl of ABTS.+ solution and 50 µl of BHT. As for the antiradical activity, ABTS scavenging ability was expressed as IC_{50} (µg/ml). The inhibition percentage of ABTS radical was calculated using the following formula.

2.7 Nitric oxide scavenging activity

Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide (NO), which cooperates with oxygen to deliver nitrite particles, which can be assessed utilizing Griess Ilosvay response[30]. Scavengers of NO compete with oxygen, leading to reduced production of NO and a pink coloured chromophore is formed. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions.

2.8 Inhibition Percentage Calculation

Percentage of inhibition was calculated from the equation

[(Absorbance of control - Absorbance of test)/ Absorbance of control)] × 100.

IC_{50} value was calculated using Graph pad prism 5.0.

Where A0 is the absorbance of the control, and A1 is the absorbance of the sample.

3. RESULTS AND DISCUSSION

Some of the plants are one of the major sources of antioxidants, anti-arthritic and anti-inflammatory agents [31,32]. The extraction was carried out for *S. alata* leaf with different solvent systems like (Chloroform, Ethyl acetate, Ethanol, Methanol and hydroalcoholic extract). The extractive yield percentage for the *S. alata* leaf extracts are (8.23%, 15.68%, 19.54%, 29.31% and 41.28%). The hydroalcoholic extract of *S. alata* showed the maximum yield percentage when compared to other extracts. On the basis of results obtained from the preliminary qualitative phytochemical analysis the hydroalcoholic extracts showed better activity when compared to other extracts. The phytochemical investigation revealed the presence of various secondary metabolites such as alkaloids, flavonoids, steroids, Terpenoids, Anthraquinone, phenols, Saponin, tannin and carbohydrates. These tests revealed the presence of various bioactive secondary metabolites which might be responsible for their medicinal properties.

3.1 Quantitative phytochemical Analysis

The Quantitative phytochemical Analysis was carried out in hydroalcoholic extracts of S.alata. *S.alata* hydroalcoholic extract contains alkaloids (49.20%), Flavonoids (6.96%) and Phenols (69.72%) (Table 1, Figure 1). A large number of these mixtures have been displayed to deliver strong hypoglycaemic, antihyperglycemic, and glucose suppressive exercises [33]. These impacts may be accomplished by working with insulin discharge from beta pancreatic cells, hindering glucose retention in gut, invigorating glycogenesis in liver as well as expanding glucose use by the body [34,35].

Table: 1 Quantitative Phytochemical Analysis	
Phytoconstituents	***Senna alata* (Leaf) Hydroalcoholic Extract (%)**
Alkaloids	49.2
Flavonoids	6.96
Phenols	69.72

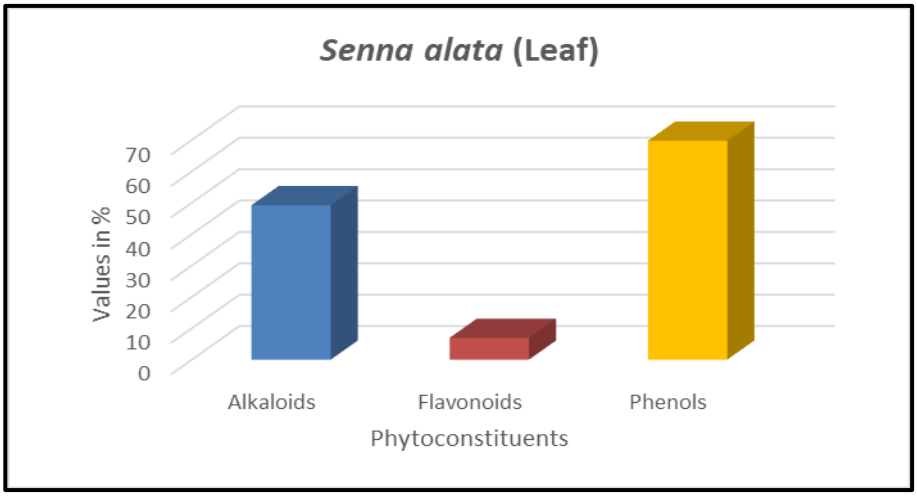


Fig: 1 *Quantitative Phytochemical Analysis*

Alkaloids are a class of basic substances which principally contain fundamental nitrogen molecules. Recognized their pain-relieving impacts[36]. The plant alkaloids can be utilized in medication as sedative specialists[37]. When controlled by creatures, alkaloids have significant physiological impacts and hence their far and wide use in medications for drug creation[38]. Phytochemical screening is a compelling strategy for recognizing new wellsprings of mixtures in plant concentrates, and is of restorative and modern significance like alkaloids, flavonoids, phenolics, chemicals, tannins, saponins, and so forth they start from any piece of the plant, like bark, leaves, blossoms, seeds. Information on the plant's synthetic constituents is significant for the revelation of restorative medications, and furthermore in light of the fact that such information might uncover new wellsprings of such rewarding materials as tannins, oils, gums, and forerunners for orchestrating complex compound substances. Likewise, information on the substance constituents of plants will likewise be basic in the disclosure of the legitimate worth of society cures[39].

3.2 Physico chemical analysis

The further studies were carried out in hydroalcoholic extract of *S.alata* as it shows more activity when compared to other extracts. Physicochemical parameters of the exact compliance with those mentioned in Ayurvedic Pharmacopoeia of India.

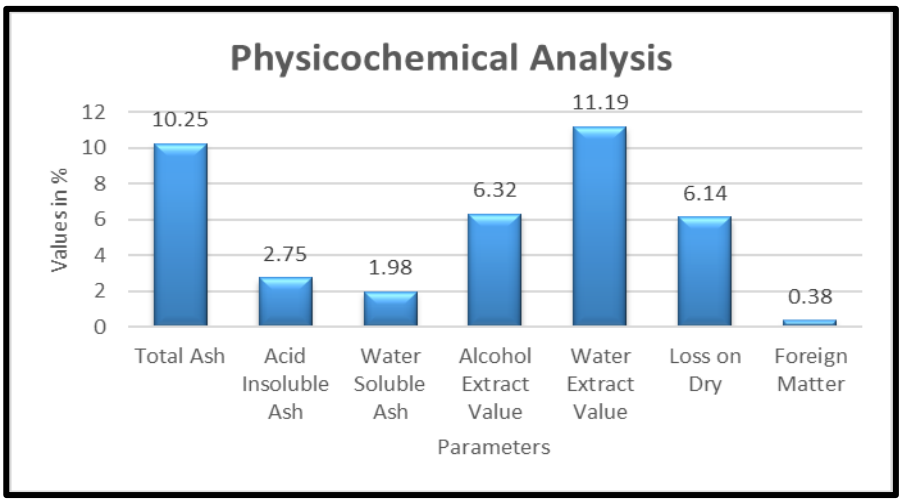


Fig: 2 *Physicochemical Analysis of Senna alata (Leaf) in Hydroalcoholic Extract and powder sample*

The percentage of Total Ash (10.25 ± 0.04), Acid Insoluble Ash (2.75 ± 0.03), Water Soluble Ash (1.98 ± 0.02) in the powder sample and Alcohol Extract Value (6.32 ± 0.03), Water Extract Value (11.19 ± 0.02), Loss on Dry (6.14 ± 0.03) and Foreign Matter (0.38 ± 0.01) (Figure 2) in the extract were determined. Fluorescence behaviour of sample were tested in Visible Light,

Short UV (254NM) and Long UV (365NM) for different parameters such as Powder (P), Powder + 1N NaOH in Methanol, Powder + 1N Hcl, Powder + HNO_3 (1:1), Powder + H_2SO_4 (1:1), Powder + 50% H_2SO_4 and Powder + 50% HNO_3 (Table 2).

Table 2 : *Fluorescence behaviour of powder with different reagent*

S.No	Parameters	Visible Light	Short UV (254 NM)	Long UV (365NM)
1	Powder (P)	Dark Green	Green	Light Green
2	Powder + 1N NaOH in Methanol	Green	Green	Light Green
3	Powder + 1N Hcl	Light Green	Pale Green	Light Blue
4	Powder + HNO_3 (1:1)	Dark Green	Light Green	Pale Green
5	Powder + H_2SO_4 (1:1)	Dark Green	Green	Pale Green
6	Powder + 50% H_2SO_4	Pale Green	Dark Green	Dark Green
7	Powder + 50% HNO_3	Pale Colour	Pale Green	Light Green

The heavy metal analysis of *S.alata* (Leaf) in sample of the elements which are present are Iron (Fe) is 4.46ppm, Copper (Cu) is 0.167ppm, Manganese (Mn) is 0.438ppm, Zinc (Zn) is 0.557ppm, Nickel (Ni) is 0.071ppm, Cobalt (Co) is 0.038ppm, Lead (Pb) is 0.268ppm, Aluminium (Al) is 6.794ppm, Vanadium (V) is 0.153ppm, Chromium (Cr) is 2.186ppm, Molybdenum (Me) is 0.714ppm, Mercury (Mg) is 0.001ppm, Arsenic (As) is 0.002ppm and Cadmium (Cd) is 0.022ppm. The results of heavy metal analysis of the powered sample reported within the WHO's recommended limits and leads to discovery of potential drugs.

3.3 In-vitro Antioxidant activity

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders[15]. Cancer prevention agents' physiological capacity is to keep away from harm to cell structure coming about because of synthetic responses including free revolutionaries. Oxidative pressure might be significant in infection pathophysiology, including atherosclerosis, persistent renal disappointment and diabetes mellitus. A free revolutionary might be an individual sub-atomic species having an unpaired electron in a nuclear orbital[40].

3.4 Reducing power assay

The presence of the reluctant in the arrangement causes the decrease of the Fe3+/ferricyanide complex to the ferrous structure[41], Thus Fe+2 can be observed by observance estimation at 700nm which is used in the prevention of cancer. Concentrate ability to lessen Fe+3 to Fe+2 (decreasing impact) was determined utilizing the strategy expressed[28]. A compound's diminished capacity can fill in as a significant marker of its potential cancer prevention agent action [42]. Examination was made with the dietary cancer prevention agent, for example, ascorbic corrosive. Diminished force compounds propose that they are contributors of electrons and can limit the oxidized intermediates of lipid peroxidation cycles to fill in as essential and auxiliary cancer prevention agents[43]. The convergence of restraint of hydroalcoholic separate *S.alata* (715.26µg/ml). In *S.alata* the least fixation is viewed as showing more grounded activity.

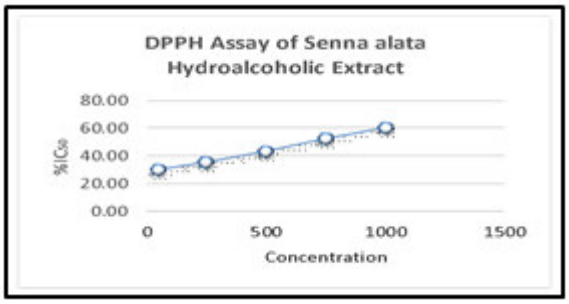
3.5 DPPH assay

Antioxidant is the compound which delays or inhibits the damage to oxidative. The most probable antioxidant defense mechanism is the direct interaction between the extract (or) compounds and hydrogen peroxide, rather than altering the cell membranes and limiting. DPPH is a stable, radical-based nitrogen used conventionally to test the free radical scavenging of antioxidants in plant extract or synthetic compounds [44]. The reduction potential of DPPH radical is determined by the reduction in absorbance at 517 nm caused by an antioxidant. The convergence of restraint of hydroalcoholic separate *S.alata* (715.26µg/ml). *S.alata* shows more activity in hydroalcoholic extract where lowest inhibition shows better activity (Figure 3).

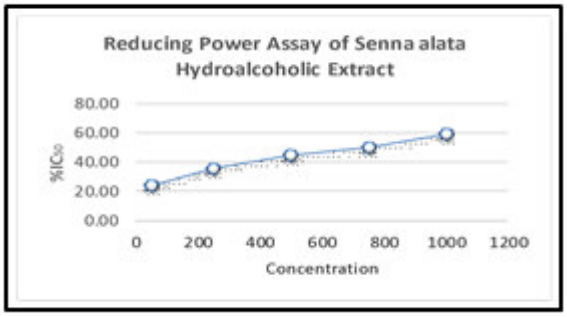
3.6 ABTS Assay

In its free structure the 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic corrosive (ABTS) radical cation is steady. Cell reinforcement move was made with 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic corrosive (ABTS) revolutionaries. Various trials and studies were taken place for ABTS assay as free radicals as huge part in malignancy, coronary illness, cancer property[45].

Reducing Power Assay



DPPH Assay



ABTS Assay **Nitric Oxide Assay**

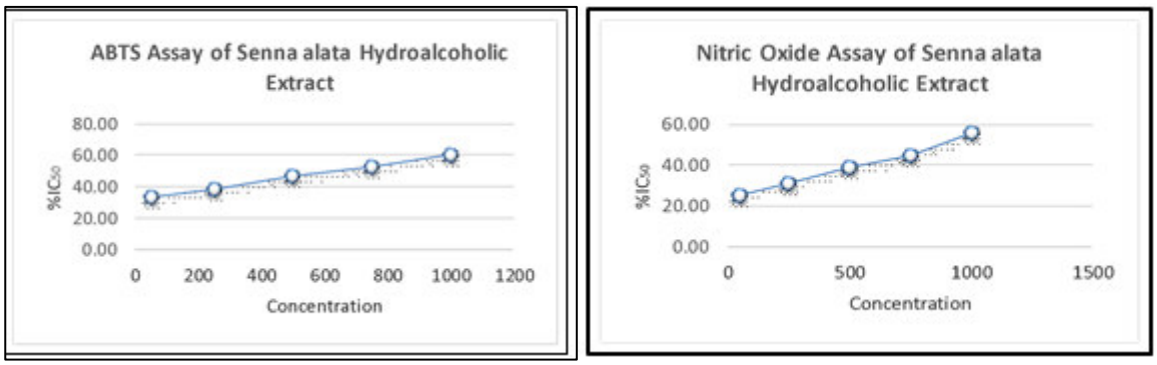


Fig: 3: *In-vitro* antioxidant activity of *S. alata* Hydroalcoholic leaf Extract

Free radicle antioxidant activity sounds as cancer preventing agents which suppress the oxidation levels and protects cells from external environments[45]. Cell reinforcement natural items are utilized to forestall and treat oxidative pressure related illnesses like diabetes, Alzheimer's sickness, atherosclerosis, stroke and malignancy [46,47]. Concentrate ability to radical cation ABTS was portrayed in Figure. Strong, focus subordinate, ABTS revolutionary cation radical movement showed in the hydroalcoholic extract. Hindrance convergences of *S.alata* (643.95µg/mL) had radical movement on ABTS.

3.7 *Nitric oxide assay*

The minimum *in vitro* nitric oxide scavenging activity of the plant extract was 25.17% at 50 µg concentration, whereas the maximum *in vitro* activity was 55.78% at 1000 µg concentration. The percentage inhibition was increased with increasing concentration of the extract. The concentration of inhibition of hydroalcoholic extract *S. alata* (856.79µg / ml). In *S.alata* the lowest concentration is seen as showing stronger operation. The decreasing limit of cancer prevention agents is because of their electron move property, for example, polyphenols flavonoids[48]. Many investigations showed that the plants extricate have a solid decreasing limit. In other hand, numerous analysts have broadly detailed the connection between polyphenol structure and their and ferric decreasing limit [49]. Phenolic compounds are called cancer prevention agents in view of their capacity to rummage free extremists, lipid peroxidation hindrance, and decreasing impact [13,50]. The study was concluded with the preliminary quantitative phytochemical, physicochemical and *in-vitro* antioxidant activity of hydroalcoholic extract of *Senna alata*.

4. CONCLUSION

The outcomes affirm that the hydroalcoholic of *S. alata* has higher activity when compared to other extracts. The phytochemical activity shows highest phytoconstituents when compared to other extracts. The preliminary analysis and physicochemical analysis were carried out for the powered sample of *S. alata*, it shows better activity and it also fulfils the WHO norms. Further activity is carried out with the *S. alata* hydroalcoholic extracts in different antioxidant activity DPPH assay shows (715.26µg/ml), ABTS assay shows (643.95µg/mL), Reducing power assay has (715.26µg/ml) and nitric oxide assay has (856.79µg / ml). The lowest inhibition shows better activity, where ABTS assay shows the lowest inhibition. The hydroalcoholic concentrate might be a decent wellspring of minerals to treat a number of infections that are for the most part caused because of the insufficiency of those minerals and can be used in Ayurvedic framework to fix sickness.

5. AUTHOR'S CONTRIBUTION STATEMENT

Mrs. Rajendran Raja Priya conceptualized and discussed the methodology and results in the final manuscript and also analysed the Results. All the author's read and approved the final version of the manuscript.

6. ACKNOWLEDGMENTS

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7. CONFLICT OF INTEREST

Conflict of interest declared none.

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